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ANNUAL REPORT

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MENTAL HEALTH INTRAMURAL RESEARCH PROGRAM-
Division of Clinical and Behavioral Research
Division of Biological and Biochemical Research
Division of Special Mental Health Research
NATIONAL INSTITUTE OF MENTAL HEALTH

July 1, 1976 - September 30, 1977

VOLUME I - SUMMARY STATEMENTS

ANNUAL REPORT *of program activities*

MENTAL HEALTH INTRAMURAL RESEARCH PROGRAM -
Division of Clinical and Behavioral Research,
Division of Biological and Biochemical Research, and
Division of Special Mental Health Research

US NATIONAL INSTITUTE OF MENTAL HEALTH

July 1, 1976 - September 30, 1977

VOLUME I

Summary Statements

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 Division of Clinical and Behavioral Research,
 Division of Biological and Biochemical Research, and
 Division of Special Mental Health Research
 NATIONAL INSTITUTE OF MENTAL HEALTH

July 1, 1976 - September 30, 1977

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ANNUAL REPORT
of the
DIRECTOR, MENTAL HEALTH INTRAMURAL RESEARCH PROGRAM
NATIONAL INSTITUTE OF MENTAL HEALTH
July 1, 1976 - September 30, 1977

John C. Eberhart, Ph.D.

The story of this year is in the reports to follow in which the Chiefs of our intramural basic Laboratories and clinical research Branches summarize the scientific events of the past 15* months. I will not further summarize that material here. The efforts of our scientists during this period continued generally at an outstanding level of effectiveness, despite the shortage in staff and money which have characterized NIMH research programs for several years. The comments in this Annual Report by Dr. Cohen on the logic of increased support of research, and by Dr. Wyatt, on the rhetoric of enhanced support, are both highly relevant.

It is a pleasure to report the award this year of the McAlpin Award by the Mental Health Association to Dr. William E. Bunney for his distinguished scientific contributions to understanding manic-depressive illness.

In the ordinary course of an average year, as I have commented in other years, those of us who run programs like this one tend to get preoccupied by administrative matters. It is hard to stay close to the science when matters of budget, space, positions, regulations, accountability, liaison with higher echelons and with cognate programs, and the like, flood in by memorandum, telephone call, visitor, and meeting. This year we tried to redress the balance a little, in two ways. At Dr. Cohen's suggestion we set aside every Tuesday morning for a scientific reporting session between the three Division Directors and relevant administrative officers and three members of the research staff. Since we often talk with Laboratory Chiefs, we left them out, and instead saw and heard in the course of the year all other permanent or tenured scientists and a few of the Senior Staff Fellows and Associates. Generally the three scientists selected were from different Laboratories, sometimes they had not previously met one another, and occasionally they discovered common interests. The format of the sessions were quite informal, generally without slides, with each scientist given 35 to 40 minutes to describe his work in any way he wished. The sessions served a valuable purpose in acquainting us in some detail with the work of scientists with some of whom we have too little contact, and in bringing together people with common interests.

The other approach, also suggested by Dr. Cohen, was that the regular Laboratory Chiefs' meetings be limited to one a month, and that the other monthly

*Though Fiscal Year 1977 began October 1, 1976 and ends September 30, 1977, the present annual report is being written to cover the preceding 3 months as well. That "transition quarter," officially part of Fiscal Year 1976, bridged the gap between the Federal Government's last traditional fiscal year and the start of a new-style fiscal period which we will have from now on.

meeting be devoted to a report on research by one of the Laboratory Chiefs. - This was agreed to and a committee chaired by Dr. Evarts arranged a schedule and stage-managed the seminars at the Faculty Center of the Foundation for Advanced Education in the Sciences (formerly the PHS Club). Refreshments added to the pleasant character of what turned out to be a useful and informative series of meetings.

This annual report is a kind of year-end journal in which I try to note the events of the year that should be remembered or should be recorded for later recall. As one looks back on the year he is conscious of the fact that the year began with the election of Governor Carter as President over Mr. Ford, that Mr. Joseph Califano was shortly named Secretary of the Department of Health, Education, and Welfare, and that other more or less expected changes took place in the upper level staff of the Department. In "H", as we now refer to the conglomeration of health agencies in the Department, Dr. Theodore Cooper was relieved from duty as Assistant Secretary for Health and Mr. James Isbister was similarly relieved as Administrator of ADAMHA. Several other agency heads were also displaced. The Secretary chose not to remove, but gave a vote of confidence to, Dr. Donald Fredrickson, Director of the National Institutes of Health, to the applause of those gathered in the Masur Auditorium to hear the Secretary, and to the cheers of the rest of the NIH. The departures of Dr. Cooper and Mr. Isbister were generally regretted, since both had performed with distinction and were devoted professionals of high competence. Successors to these two have been Dr. Julius Richmond as Assistant Secretary for Health, and Dr. Gerald Klerman, designated to be Administrator of ADAMHA. Both have fine backgrounds and many friends here, and we wish them well. I would like to note here with admiration and gratitude the fine job Dr. Neil Waldrop has done and (in October, 1977) is still doing in the difficult role as Acting Administrator of ADAMHA.

Sometimes a journal of events should also record some non-events, and I would like to mention two. This was a year when there was no reorganization affecting the Intramural Program, and when there was no increase or decrease in our research space. Both deserve a little comment.

Interest in reorganization was stimulated last year by the President's Biomedical Research Panel, almost half of whose members believed that ADAMHA's institutes, or at least their research programs, should return organizationally to the National Institutes of Health. Though this idea continues to be talked about, especially in the light of ADAMHA's building needs and the possibility of a site on the NIH campus, there is no real indication that the administration is contemplating such a change. So organizationally we had a peaceful year.

The research space situation has several aspects worth mentioning. 1) The Ambulatory Care Research Facility is now under construction, with an enormous excavation extending across the entire front of the Clinical Center. We have had a busy year working out plans for moves made necessary by the construction, especially for relocating Ward 2 West, which will need to be vacated by us about a year from now. Turn-around space for both laboratories and patients is severe and perfect solutions are not likely to

be found for all our needs. 2) The Laboratory of Socio-Environmental Studies will move within the next two or three months to new space in Building 31. It finally became apparent to all of us that it would be hard to justify the indefinite continuance in Building 10 of a Laboratory which did not work with patients and did not use the wet laboratory facilities, despite the program value of having easy communication between scientists in all laboratories. This laboratory has been in Building 10 since it opened in 1953, and it is with regret that we contemplate the necessary move 200 yards down Center Drive.

Also moving into Building 31 at the same time will be most of the Laboratory of Psychology and Psychopathology. They will retain a small amount of experimental space in Building 10 for work with patients, but are moving out of the second floor of Building 10 to accommodate certain necessary moves of Clinical Center staff incident to ACRF construction. This Laboratory is expected to stay in Building 31 until the ACRF is completed and some remodeling is done in the Clinical Center. Then they will move back, probably in 1981.

A part of the space story is the search for new research facilities for those intramural programs in ADAMHA now inadequately housed. I summarized the start of this a year ago. The need for more space is acute for our Division of Special Mental Health Research, now located in the William A. White Building at St. Elizabeths Hospital, and which should be relocated on the NIH Campus near the main body of our Intramural Program. The National Institute of Drug Abuse needs to find a new permanent home for the Addiction Research Center; tentative plans have been made for a move of that unit to remodeled laboratories in Baltimore. The National Institute of Alcohol Abuse and Alcoholism also has a laboratory in the White Building, is planning to move it soon to rented space in Rockville, and wishes to consolidate that unit and its other two planned laboratories in a new facility, preferably at NIH. The study by the Arthur Young Company entitled, "Review of Intramural Research Facilities," was submitted on June 17, 1977. It outlined the facilities now used for intramural research by the three institutes, reported their estimates of future needs, and outlined various options for meeting the needs. The options differed principally in the site of a new research facility—NIH or elsewhere—and in the units proposed for housing there. Several options proposed that all NIMH Intramural Research be moved to such a new facility, including the two Divisions now housed at NIH. I have declared these options unacceptable. It would be a serious disservice to our Bethesda programs to move them away from their close association with NIH research programs in the Clinical Center and Building 36.

New space is needed for the programs mentioned above, and I hope that a way will be found to build new space at NIH that can accommodate the Division of Special Mental Health Research, the Addiction Research Center of NIDA, and the NIAAA intramural program. Within the next 5 to 10 years Building 9 will probably be torn down, because it is inadequate for housing animals and would be uneconomical to remodel, and we will therefore need new space for the Neuropsychology Laboratory and the Central Primate Facility. Finally, the laboratories now in the Clinical Center are tightly packed, and completion of the Ambulatory Care Research Facility will not materially change that. It would be important to secure enough new space in any new construction to take care of our present program needs.

As I write this, discussions have already taken place within ADAMHA and NIH on the nature of the needs (ADAMHA) and on possibilities of meeting those needs (NIH). A meeting will soon be held between the heads of the two agencies and Assistant Secretary Richmond for a preliminary exchange of views on a possible new structure. The fact that the NIMH Intramural Program is jointly administered by the two agencies has made it appropriate for me to participate in the deliberations of both agencies. Though there is some potential conflict in that role, I sense so far some smoothing of the way.

A year ago I commented at some length on the Presidents Biomedical Research Panel, which had turned in its final report in April, 1976. This year one of the early acts of President Carter was to appoint a President's Commission on Mental Health, under the chairmanship of Dr. Thomas Bryant, and with Mrs. Rosalynn Carter as Honorary Chairman. Operating under the panel were two dozen Task Panels, one of which dealt with research. Its coordinator was Dr. Daniel X. Freedman, and it included such friends and present or former associates as Seymour Kety, Floyd Bloom, Norman Garnezy and others. The Research Task Panel submitted its Preliminary Report on September 1. The final reports of both are due April 1, 1978.

The Task Panel Draft Report on Research reviews major current problems in mental health research, both as subject matter and as to funding, emphasizing the real decrease (in constant dollars) of NIMH research appropriations over the past few years. It recommends that the 1979 NIMH research budget be increased by 20 percent over the 1978 figure, and the Commission endorses this recommendation. The Research Panel further urges that more support in 1978 be given to "fundamental bio-behavioral and social sciences" than has been the case in the recent past, because "the fundamental sciences have become woefully undersupported." It urges an increase in research training by NIMH, an increase in involvement by minority students, better support of research training for psychiatrists, and improved administration of the Research Scientist Award Program.

The final report of the Panel will deal more fully with a number of matters of direct interest to the NIMH and Intramural Research Program. Among them are expected to be 1) "a description and . . . an examination of the anatomy of the mental health research institutes," including "how research is organized, where it takes place, who carries it out, and who funds it"; 2) an assessment of the "state of the art" of mental health research; 3) a critique of "the research process and the organization and administration of research The organizational priorities and issues of existing research review and advisory process, intramural versus extramural research, desirability of research centers, intra-agency duplication, and service/research interaction will be examined . . ."; 4) analysis of some clinical research issues, such as "the role of clinical centers, insurance reimbursements, the costs associated with clinical research, and . . . the sometimes blurred boundaries [of] clinical, applied, and basic research, clinical trials, demonstrations, and program evaluation"

All of these issues are of concern to the NIMH and the Intramural Research Program, and we are looking forward to the findings and comments of the Task Panel in their report.

The strength of the Intramural Research Program depends not only on money and space, which have been mentioned, but on positions. In a sense, the Intramural Research Program uses people where the Extramural Research Program uses dollars. We have had a continuing but uneven loss in budgeted positions for about the last ten years, for a total loss of about 20 percent. This note is to report a further loss this year. We hope it will turn out to be temporary. The new administration froze all "vacancies" existing on February 28, 1977, and thereafter permitted the filling of only 3 out of 4 new vacancies that developed, until about July 1. Although we had recruitment actions under way for all the vacancies we had on February 28--normally we are permitted to fulfill such understood commitments--the rules were changed this time and our requests were denied. As a result we now (October 21) have 25 positions vacant out of our normal ceiling of 373. The Institute has fortunately retained its ceiling of 893, and we hope the Intramural Program will also retain its ceiling. It will be a serious blow if it does not.

The Board of Scientific Counselors met twice this year, as usual. On November 4-5, 1976 the Board reviewed the Laboratory of Clinical Science. In addition to members of the Board (Drs. Robert Doty, Eleanor Gibson, Nelson Goldberg, David Hamburg, Eleanor Sheldon, and Frederic Worden) the following consultants assisted in the review: Dr. Sydney Spector, Roche Institute of Molecular Biology; Dr. Donald Jenden, UCLA; Dr. Eugene Roberts, City of Hope Medical Center; and Dr. Norman Weiner, University of Colorado.

On March 24, 25, and 26, 1977, the Board convened again in Bethesda to review three Laboratories: Neurobiology, Neurophysiology, and Cerebral Metabolism. Ad hoc consultants for this occasion were Drs. Bernard Agranoff, University of Michigan; David Carpenter, Armed Forces Radiobiological Research Institute; Sidney Ochs, University of Indiana; Fred Plum, Cornell; and Marion Smith, Palo Alto Veterans Administration Hospital.

Evaluations of the work of all four Laboratories were, we were pleased to hear, distinctly favorable. Since it seems inappropriate to condense their comments enough for this space, I will let it go with that. I would, however, like to record my admiration for the comprehensive, thorough, and serious way in which the Board has carried out its responsibility to review and appraise these comparatively large and complex programs. It is a major service to us, and it requires a concentration of effort which is beyond that required in most consultant roles.

This was the final year on the Board for two members, Drs. Eleanor Gibson and Eleanor Sheldon. We are sorry to lose them and we thank them for their notable contributions. I want to say a special word of appreciation to Dr. Sheldon, who for the past two years carried the responsibility of Chairman. The fact that she was a sociologist in a heavily biomedical program, did not faze her in the least, and she served with great effectiveness.

Our major personnel loss during the year was the retirement of Mrs. Margaret Braymer as Chief of the Satellite Personnel Office in August, 1977. I have commented on her outstanding value to this program in several of these annual reports, and I will say here only that I do not expect to see her equal for a long time. We are fortunate to get Mr. Barton Kuhns as her

successor. He had the benefit of working with Mrs. Braymer for several years, and we fully expect him to make contributions of his own distinct sort as he works into his new job.

Finally I would like to mention that Dr. Richard J. Wyatt, Chief of the Laboratory of Clinical Psychopharmacology in the Division of Special Mental Health Research, has agreed to take on the added duty of Director of the Division. He has served as Acting Director for the two years since Dr. Floyd Bloom left, and he now becomes the regular Director. It complicates his life in many ways, and may not add to his satisfactions, and we are therefore more than grateful that he has been willing to take on the responsibility.

Annual Report of the
Director, Division of Clinical and Behavioral Research
National Institute of Mental Health

July 1, 1976 - September 30, 1977

Robert A. Cohen, M.D., Ph.D.

In my annual report last year I spoke of some impending changes in the Clinical Center, both organizational and structural. The implementation of these changes has begun. Clinical Center administration has drawn closer to the operation of the individual Institute research programs, and in so doing has become more responsive to our needs and problems as they develop. The Center is beginning to function more as a total hospital rather than a group of individual fiefdoms. While there were advantages to be gained from the old arrangement, the new procedures have brought new strengths to the setting in which we work; they augur well for the future. The construction of the new Ambulatory Care Research Facility has begun; we have already experienced some expected and also some unanticipated inconveniences to which we will have to adjust during the next few years.

The reports of the laboratory and branch chiefs describe in detail the wide range of studies which are being carried out. In our view, and in the view also of our Board of Scientific Counselors, the work is generally at a high level. Dr. William E. Bunney was given the McAlpin Award by the Mental Health Association for his outstanding contributions to the understanding of manic-depressive illness. In recognition of their notable contributions in related areas, the sections headed by Drs. Frederick Goodwin and Dennis Murphy were raised to Branch level--the former being designated as the Clinical Psychobiology Branch, the latter as the Clinical Neuropharmacology Branch. Concurrently, the name of the Adult Psychiatry Branch was changed to the Biological Psychiatry Branch; this reflects more accurately the character of its interests. Under the leadership of Dr. Bunney, who also served as Deputy Clinical Director, the Clinical Investigations Coordinating Committee has made considerable progress in establishing certain uniform data-collecting and patient-care procedures followed on all five of our patient units. These have already significantly strengthened the clinical program; they promise to provide a storehouse of highly reliable data which will be an invaluable and unique asset in our future work. Over five patient units are operating at a higher level of productivity and efficiency than ever before, but at the same time are held back from operating at optimal levels because of a shortage in supporting staff.

A less happy development was the request of Dr. David Rosenthal to give up his responsibilities as Chief of the Laboratory of Psychology and Psychopathology in order to devote full time to his research on genetic aspects of mental illness. Dr. Rosenthal had served as Chief of the Laboratory of Psychology since 1966. We accepted his decision with regret, and plan to appoint a search committee to seek a new Chief as soon as we can secure definite information concerning personnel ceilings and the level of the resources which will be available.

In the last report I also expressed the hope that the Report of the President's Biomedical Research Panel and the many formal review procedures which have been instituted from on high to strengthen the administration of research would have a stabilizing effect. This happy state has not yet been achieved. We continue underbudgeted and understaffed. It is not too difficult to see how such a simple and direct statement has failed to impress those who must make the final decisions concerning the level of support afforded us. On the one hand, we assert that the work is going better than ever; on the other, we seek more resources in these troubled times when the Congress and the Administration are besieged by desperate petitioners. In my view, our difficulties arise from the fact that for the past ten years our support has not been stable, but has eroded at a slow but inexorable rate. Our investigators each year must stretch a bit farther to reach their goals. Despite their notable achievements, they are in a position where a ten percent increase in support might well lead to a twenty or thirty percent greater pay-off. At the same time, a further reduction might force constriction or even cancellation of a study which has high promise. Modern science does not stand still. New instrumentation has ten times the sensitivity of that currently in use. And so, scientists in the most powerful research institute in the world find themselves driving Model Ts past showrooms displaying Aston-Martins. One can still explore much of the countryside in a Model T, but the eminence the intramural programs at NIH enjoy today was not achieved, nor is it likely to be indefinitely maintained, if this proves to be a final decision.

A recent issue of *Daedalus* which examines health policy in the United States is subtitled "Doing Better and Feeling Worse." The mordant quip refers to the fact that although health expenditures increased from 10 billion dollars in 1950 to 130 billion in 1975, they did not result in better health. In his paper, Aaron Wildavsky points out that "the medical system affects about 10 percent of the usual indices for measuring health: whether you live at all (infant mortality), how well you live (days lost to sickness), and how long you live (adult mortality). The remaining 90 percent are determined by factors over which doctors have no control, from individual life style, to social conditions, to the physical environment. Most of the bad things that happen to people are at present beyond the reach of medicine. In the absence of medical knowledge gained through new research, or of administrative knowledge to convert common practice into best practice, current medicine has gone as far as it can. It will not burn brighter if more money is poured on it. No one is saying that medicine is good for nothing, only that it is not good for everything."

It seems to me that the entirely natural and eminently reasonable demands for improved distribution of improved services have resulted in an attenuation of research support. Yet it is only by further research that these heavy service burdens can ever be lightened, that prevention of illness can ever be achieved. If those who demanded service at the expense of research had prevailed in the case of poliomyelitis, we would today have thousands of iron lungs instead of an effective vaccine. It is not unreasonable to be asked "Can't you do more with what you already have?", or to be visited by experts who propose to help us improve our effectiveness as managers. We shall continue to strive for such improvements, but they alone will not be sufficient.

Annual Report of the
Acting Director, Division of Special Mental Health Research
National Institute of Mental Health
July 1, 1976 - September 30, 1977

Richard J. Wyatt, M.D.

While the quality of the work produced by the three laboratories over the past year continues to be high, the Division's difficulties concerning staff turnover and location have persisted. The Laboratory of Neuropharmacology has had three Acting Chiefs since Dr. Bloom left. Dr. Siggins and Dr. Hoffer each held this position for three to four months before their previously planned departures to the Salk Institute and the University of Colorado respectively. At present, I am serving as Acting Chief while a committee looks for a head for the laboratory. Despite these problems and although the scope of this laboratory has become somewhat limited, Drs. Weight and Taylor and Mr. Oliver and Mrs. Krauthamer have maintained the scientific excellence of its work.

The Laboratory of Preclinical Pharmacology has continued its enormous qualitative and quantitative productivity despite a medical problem of its Laboratory Chief, Dr. Costa, late last year. Dr. Costa has fully recovered. He has decreased some of his outside commitments but has continued his vigorous and exciting laboratory work.

My own laboratory - the Laboratory of Clinical Psychopharmacology - has continued its efforts toward understanding sleep, drug abuse and schizophrenia. One exciting finding is that recently we have been able to distinguish some paranoid from nonparanoid schizophrenic patients using platelet monoamine oxidase.

The relationship of our Division to the Hospital (which was the subject of last year's report) has improved on one level. The Hospital has clearly attempted to make it easier for us to function. In return, we are increasing our efforts to provide the Hospital with specialized abilities that we possess.

However, problems do remain. We continue to have frequent power outages which have ruined numerous experiments and have destroyed expensive equipment. The basic utilities provided to our building are antiquated and simply not designed for modern research use. They fail at an unfortunately predictable rate and get patched together with whatever can be devised. This band aid approach will collapse. Without question, medium to long range plans for the Division are still necessary.

State of Research Funding

During the past year, I have had considerable contact with several committees of the Mental Health Association. They have wanted to better understand our research in the IRP and how it might eventually effect mental illness. They are well aware of the cost of mental illness in human terms, but find it difficult to communicate this to Congress and others who make policy decisions. In order to help them, we have compiled summaries of mental health statistics.

The numbers of people requiring care for schizophrenia are large. One percent of the nation has schizophrenia and two or more percent will have a schizophrenic-like illness at some time during their lives. In certain urban areas this prediction rises to as much as six percent, more than one in 20 persons. One-fourth of all hospital beds and one-half of all mental hospital beds are occupied by schizophrenic patients. Sixty-two percent of schizophrenic patients require inpatient care after their first hospitalization and only 20 percent work compared to 55 percent of other psychiatric patients.

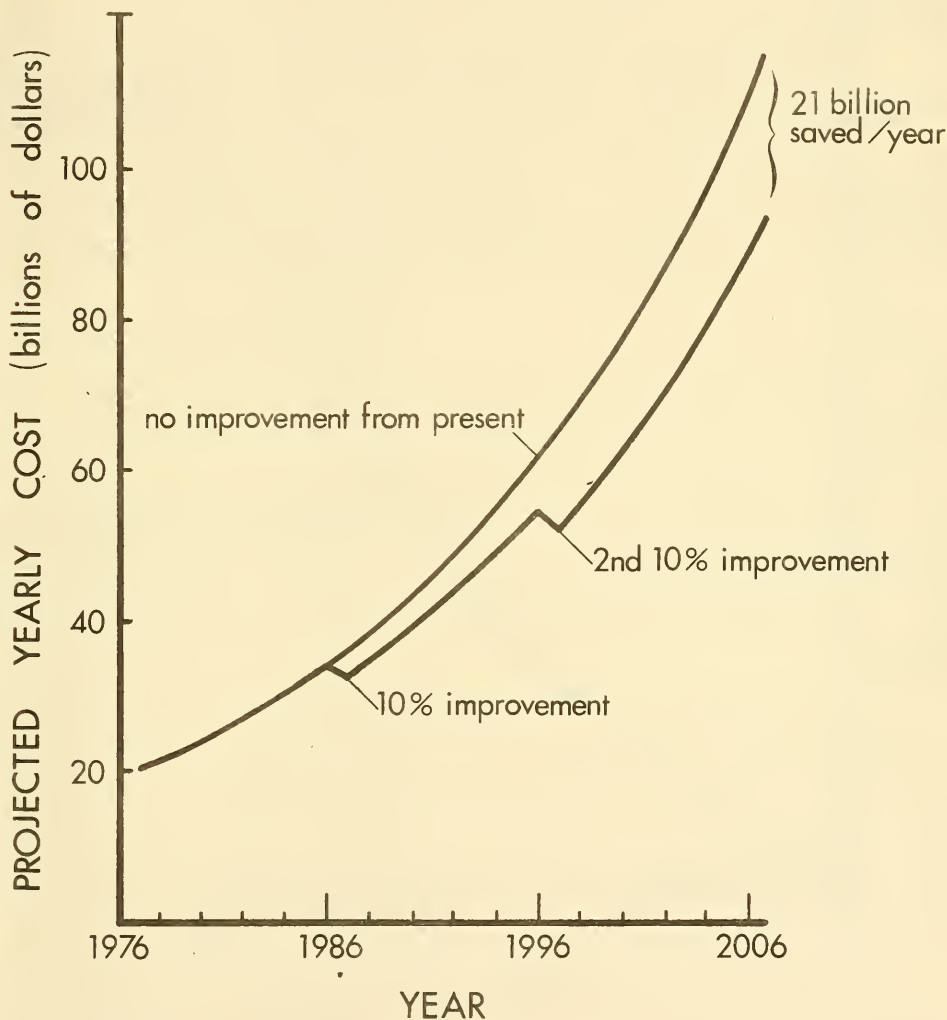
The cost of schizophrenia is staggering. Health care and lost wages for schizophrenia equal over half the total cost for all mental illness (20 billion for schizophrenia, 36.8 billion for all mental illness). The cost of schizophrenia is slightly more than the cost of cancer at 18.9 billion. Yet, the Federal Research effort in 1976 for cancer was 600 million dollars compared to 10 million for schizophrenia. Thus, cancer spends 3.2 percent of its cost on research, schizophrenia .05 percent. For further comparison, the Defense Department spends 10 percent of its 100 billion dollar budget on research.

We present two figures projecting the cost of schizophrenia. Both figures assume that we will continue to have six percent inflation over the next 30 years; health care has actually gone up much more rapidly in recent years. Twenty-nine years (Figure 1) from now the nation will be spending 114 billion dollars on schizophrenia. The cumulative toll will be one trillion seven hundred billion dollars (Figure 2).

The second line on the figures assumes that through basic research we can prevent ten percent of schizophrenia by 1986, another ten percent by 1996. These estimates are used only as an exercise and there is no current reason to believe this can be achieved. If such assumptions are correct however, the cost of schizophrenia in 2006 will be 21 billion dollars less than it would be otherwise and the cumulative total 225 billion dollars less.

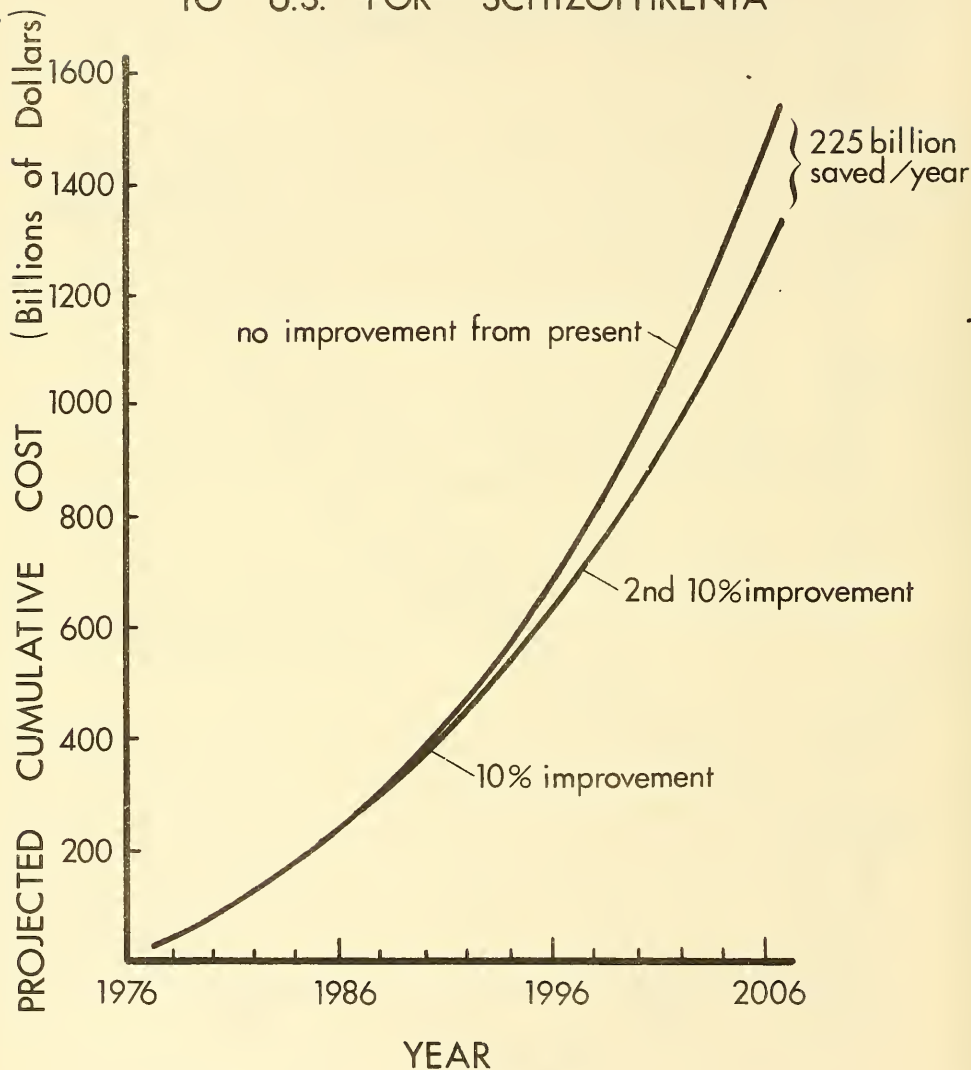
It is necessary to emphasize the importance of exploring the basic nature of schizophrenia in order to reduce its enormous cost. Maintenance therapy (the necessary alternative for a majority of schizophrenics) is extremely expensive. For example, the current cost of maintaining 50,000 kidney patients on high technology dialysis is one billion dollars. Undoubtedly, the cost of maintaining a majority of the nation's 2,000,000 schizophrenics will become higher as our technology improves. The Salk vaccine which came from a basic understanding of polio has enabled us to prevent this disease. This vaccine saved the nation six billion dollars in its first six years of use. In order to reduce the staggering cost of schizophrenia, more funds must be directed at the understanding of this disorder through basic research so that we can either prevent or cure it.

PROJECTED YEARLY COST TO U.S. FOR SCHIZOPHRENIA*



(*Assuming 6% annual inflation)

PROJECTED CUMULATIVE COST TO U.S. FOR SCHIZOPHRENIA*



(*Assuming 6% annual inflation)

ANNUAL REPORT OF THE BIOLOGICAL PSYCHIATRY BRANCH
National Institute of Mental Health
July 1, 1976 - September 30, 1977

William E. Bunney, Jr., M.D.

During the past year the name of the Branch was changed from Adult Psychiatry Branch to Biological Psychiatry Branch, which is more commensurate with the current focus of the investigations in the Branch. It also allows us to officially include the child research program which has been developed over the last few years. Many of our protocols are in the data collection phase while others have been completed and the work is in the process of being analyzed and published. However, investigations of the psychopharmacology unit, the psychogenetic unit, and the drug abuse unit are still in the initial data collection period. Approximately 60 papers were published or accepted for publication in the past year, indicating that a number of the investigations have already produced results.

The administrative and research functions of the eight sections or units in the BPB are briefly reviewed below.

During the past year, Dr. Post was named Chief of the Section on Psychobiology. Due to the volume of his publications, his contribution of a significant scientific body of information, and his administrative skills he is the first member of the Branch to be appointed as a Section Chief. In the past year the work in this Section focused on cerebrospinal fluid strategies including CSF-norepinephrine and CSF electrolytes as an approach to the study of brain mechanisms in relation to behavior. Dopamine mechanisms in mania and depression were investigated through the use of receptor antagonists such as pimozide and receptor agonists, i.e., piribedil. The effect of sleep deprivation in acutely decreasing depressive psychopathology was studied. Dr. Post has continued his work on animal models of psychopathology, focusing on electrical and pharmacological kindling phenomena.

The Section on Neuropsychopharmacology is involved in genetic, pharmacological, biochemical and psychological studies of acute schizophrenia. Dr. van Kammen heads this Unit. Investigations in this unit continue to evaluate the dopamine hypothesis of schizophrenia. A double-blind study has documented the efficacy of the specific dopamine receptor blocker, pimozide, in the treatment of schizophrenic symptomatology and has delineated an appropriate dose range. The existence of paradoxical effects of amphetamine has been shown in severely psychotic patients. A subgroup of acutely psychotic patients who remit after withdrawal from therapeutically recommended doses of neuroleptics has been identified and studied.

Dr. Gershon is Unit Chief of the Section on Psychogenetics. The goals of this Section involve the identification of biological and psychological factors that are genetically transmitted in major psychiatric disorders and the development of pharmacological and pharmacogenetic studies of psychoactive agents in well-state patients and normal controls. The biological and psychological data collection phase of investigations of hundreds of patients will

be completed within the next year. Data analysis is already in progress. A series of new pharmacological protocols for utilization in well outpatient studies are being developed by this Unit in order to search for pharmacological markers which produce a reliable psychological or biochemical change in the patient and which segregate with the illness in pedigrees.

Two additional child psychiatrists have joined the Unit on Childhood Mental Illness, Dr. Judith Rapoport and Dr. Donald McKnew. A pharmacokinetic study on utilization of amphetamine in hyperactive children has been completed by Dr. Brown and Dr. Ebert. It clearly represents the first accumulation of such data in this patient population. A double-blind high risk study of the children of parents hospitalized for affective illness is underway following an initial pilot study. Dr. Rapoport has conducted a study of the effect of amphetamine in normal children and is in the process of studying the effects of pharmacological agents in children with enuresis. The child program continues in collaboration with the LCS and Drs. Kopin and Ebert. In addition, children have been hospitalized and studied in collaboration with Dr. James Sidbury Jr., Scientific Director for Intramural Research, National Institute of Child Health and Human Development.

Dr. Glen Davis continues to head the small Drug Abuse Unit. Dr. Davis took the lead in initiating a study in collaboration with Dr. van Kammen's unit and Dr. Wyatt's program at St. Elizabeth's Hospital to investigate the effect of narcotic antagonists on mood and psychotic thinking. The results of this work, published in *Science*, demonstrate that it is unlikely that endogenous opiate-like substances play a significant role in hallucinosis and psychosis of schizophrenia. Additional work has been completed with Dr. Buchsbaum studying the mood component of the experience of pain in unipolar and bipolar depressed patients and the effect of drugs on this phenomenon. Attempts to study the effectiveness of pharmacological agents in blocking behavioral components of the "high" of various drugs of abuse continue. Although a few patients have been admitted to these protocols and have been studied, it remains difficult to recruit such patients and to conduct such studies due to the extreme unreliability of this population. At the present rate it will take four to eight years to accumulate a sufficient number of patients for these investigations.

Dr. C. Gillin and Dr. R. Wyatt are coordinating the scientific efforts of the Unit on Sleep. Dr. Sitaram has taken the lead in studying the relationship of acetylcholine to REM sleep, while Dr. Mendelsohn has investigated the relationship between biogenic amines and pituitary hormone secretion. Studies on the alterations of sleep during the switch process have continued.

Dr. Monty Buchsbaum heads the Unit on Perceptual and Cognitive Studies. During the last year the Unit has continued its work using a biochemical high risk strategy, explored the ways of relating human pain response in normals and patients to the endorphin system, and developed mathematical tools to approach measures of electrophysiological response stereopathy.

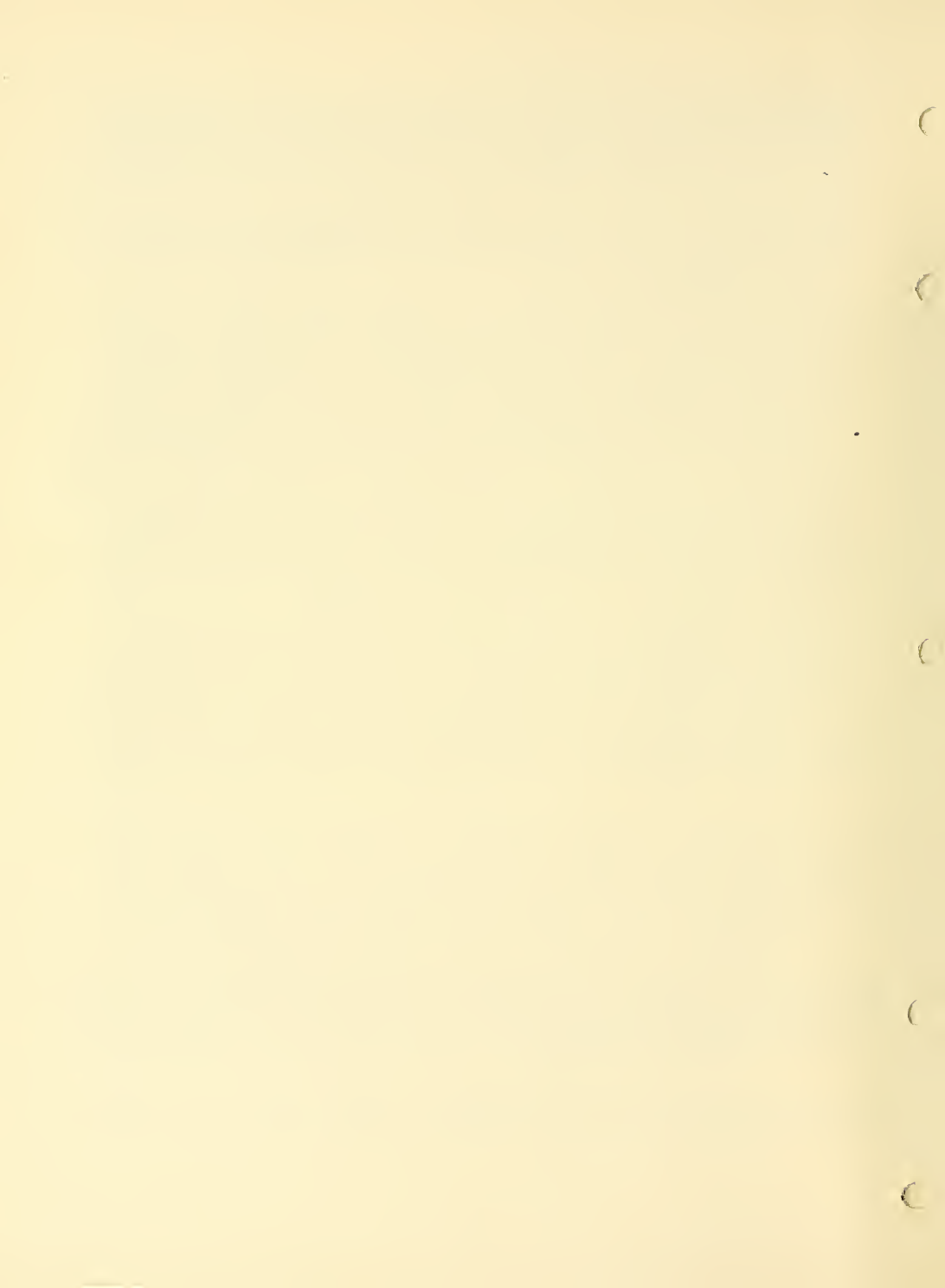
The Section on Biochemistry and Pharmacology has been extremely productive during the past year. Basic work relevant to the neurosciences has been pub-

lished by each of the five senior scientists in the Section during the past year, Drs. Tallman, C. Pert, A. Pert, Gallager and Kafka. In addition to this, each of these individuals has conducted collaborative clinical studies with each of the clinical programs. In the basic area, research continues on opiate receptors and their endogenous ligands, and on the beta adrenergic receptor and the ability of beta adrenergic agonists to activate adenylcyclase in cultured hela cells. Work also continues on studies of artificial bipolar lipid membranes. Collaborative studies with the various clinical units involve the development of assays for cyclic-AMP and GMP, gamma-aminobutyric acid, and enkephalins in CSF.

One of the most striking aspects of the current research efforts in the Branch has been the interaction and collaboration among the basic and the clinical scientists. Although I have discussed this a number of times in previous Annual Reports, and although I have seen various attempts at such collaboration, the current degree of collaboration, the ability to move rapidly and develop new assays in man which have never been utilized before, is a phenomenon that, to my knowledge, is unparalleled anywhere in the world in psychobiological research. The capability and the genuine interest of our small group of basic scientists in collaborating on clinical studies deserves unusual notice and high praise. The flexibility that it provides to move into new areas and, conversely, to give up old and unproductive areas, is critical to the best utilization of the resources of the Branch. The rigorousness of the thinking of the basic scientists concerning the design and analysis of the clinical data is also most important.

The study of receptors in animals and man continues to unite many of the investigations within the Branch. At the basic level, an understanding of receptor mechanisms is being searched for. A variety of receptors is being studied, and receptor binding techniques are being utilized. In the clinical area, theories have been developed concerning the possibility that receptor sensitivity may be altered in mania, depression and schizophrenia. Pharmacological agents which block or activate specific receptors are being used in double-blind clinical trials.

One last issue is important to note and that is the fact that the new Ambulatory Care Research Facility construction involves disruption of most of the activity on the second floor. Knowledge of this impending disruption has caused a major morale problem. The disruption of activities on the second floor will involve the BPB laboratories of the Unit on Perceptual and Cognitive Studies, the offices of the Unit on Childhood Mental Illness, most of the laboratories of the Section on Biochemistry and Pharmacology, and ward 2-West. Continuation of efforts to develop clearcut alternative sites and turnaround space for these laboratories and to develop plans so that a minimum of disruption is experienced by these scientists is, in my opinion, the most important administrative issue which faces the Branch and the Division during the coming years of construction.



Annual Report of the Clinical Neuropharmacology Branch
National Institute of Mental Health
July 1, 1976 - September 30, 1977

Dennis L. Murphy, M.D., Chief

The former Section on Clinical Neuropharmacology of the Laboratory of Clinical Science was converted to an independent Branch in February, 1977. Dennis L. Murphy, M.D. (Chief), Jonathan L. Costa, M.D., PhD. and Carol Hoover, D.S.W. are the professionals in the Branch with regular staff appointments. The clinical research program of the Branch is conducted on the 3-East patient unit in the Clinical Center. In addition to a highly capable group of clinical associates and support staff, the Branch has benefitted in the last year from two exceptional visiting staff, Dr. Paul Grof, Professor at McMaster University in Canada and Dr. Robin Murray from the Maudsley Institute of Psychiatry in London.

A major clinical research focus in the last year was a pilot crossover study comparing the effects of two monoamine oxidase (MAO) inhibiting drugs, clorgyline and pargyline. Recent developments from biochemical studies of MAO have identified two forms of this enzyme with selective effects. MAO-A preferentially deaminates serotonin and norepinephrine, while MAO-B deaminates phenylethylamine and, in human brain, dopamine. In studies in vitro, clorgyline is over 500 times more active against MAO-A than MAO-B, and pargyline has a similar preferential ability to inhibit MAO-B. In our clinical study, we have some preliminary evidence that this specificity is maintained in vivo in man. Drs. Campbell and Shilling have established assays for plasma levels of these substances. Both drugs appear to be effective antidepressant agents, but results are not yet available from other studies on sleep changes, urinary and CSF metabolites and psychological and psychophysiological measures to indicate what physiologic and behavioral consequences might attend preferential inhibition of these two forms of the enzyme. Dr. Lipper did an exemplary job as director of this project in its first year.

A second area of concentrated investigation on the clinical unit in the last year was the neuroendocrine correlates of treatment with several psychoactive drugs. These studies, which were directed by Dr. Slater, identified prolactin elevations during both clorgyline and pargyline treatment. An intensive investigation of the neuroendocrine affects of lithium administration is in the data analysis stage. These studies were accomplished in conjunction with Drs. Sklyer and Lovenberg from NHLBI. Because some of the observations from last year's study of the neuroendocrine changes with other drugs might result from changes in the sleep-making cycle, combination sleep-neuroendocrine investigations are now under way in collaboration with Drs. Gillin and Mendelson.

In other investigations accomplished collaboratively, psychological test differences between bipolar and unipolar patients studied by Dr. Donnelly with our group were found to be a replicable distinction in a new, larger patient sample. Depression-related phenomena in verbal learning and information processing studied by Dr. Weingartner with our group have been objectively

characterized. A study on the marital conflict of manic-depressive patients, as registered on the Conflict in Marriage Scale (CIMS) is being carried out by Dr. Hoover in collaboration with Dr. Roy Fitzgerald, a former clinical associate. A sample of parents of manic-depressive patients is also being evaluated using the CIMS.

Investigations of platelet monoamine oxidase in relation to psychopathology have been directed recently toward studies in normal populations -- particularly in work accomplished by Drs. Buchsbaum, Haier and Schooler. Replication of earlier work indicating associations between personality characteristics (especially sensation seeking features and tendencies towards increased psychopathology) and platelet MAO activity was successfully accomplished in a large, independent sample of college students. Studies of psychiatric patients using more refined means to characterize individuals have focussed attention on certain features of chronic schizophrenic patients such as paranoia as most closely correlated with decreased MAO activity. Biochemical studies of different human, monkey and rodent tissues have added further information indicating that MAO types A and B are separate enzymes. In parallel with the clinical study of clorgyline and pargyline on our unit, Dr. Campbell has conducted a collaborative study with NHLBI investigators of the chronic effects of these drugs in rats.

In continuing investigations of the processes regulating the dynamics of uptake, storage, and secretion of biogenic amines by human platelets, Dr. Costa has made innovative use of a wide-ranging variety of techniques. The platelet vesicular content of metal ions and phosphorous has been examined with an electron microprobe X-ray analysis system. Contrary to earlier hypothesis, it seems unlikely that calcium, phosphorous and serotonin are stored as a micellar complex, since total vesicular calcium and phosphorous do not change following large alterations in serotonin content. Based on studies with ³¹P nuclear magnetic resonance techniques, it appears that while the uptake of serotonin into vesicles may be associated with a proton gradient, the plasma membrane uptake of serotonin does not proceed by a similar mechanism. Serotonin studies made possible by Dr. Costa's thrombin-formaldehyde methodology have demonstrated that platelets appear capable of a graded secretory response rather than an all-or-none type of secretion. Plasma membrane uptake of serotonin is little affected by metabolic poisons or the presence of cytoplasmic serotonin, but is inhibited completely during the secretory response. Overall, it appears that amine dynamics in platelets are more complex than less exact techniques available for studies in other amine-storing tissues indicated. Ultimately we hope to apply these techniques to examine the effects of psychoactive drugs on aminergic function using the platelet model.

Annual Report of the Clinical Psychobiology Branch
National Institute of Mental Health
Frederick K. Goodwin, M.D.

During this last year, the Section on Psychiatry, Laboratory of Clinical Science was elevated to Branch status, and designated the Clinical Psychobiology Branch. The new Branch has been organized into four functional units:

The Clinical Research Unit, Dr. Thomas Wehr, Chief
The Unit on Neurochemistry, Dr. Paul Marangos, Chief
The Outpatient and Follow-Up Unit, Dr. Philip Gold, Chief
The Unit on Family Studies, Mrs. Yolande Davenport, Chief

These separate units have been designated to clarify lines of administrative responsibility. Scientifically, all of the units are closely integrated, and many of the individual projects involve the participation of more than one unit. Similarly, although each person in the branch is primarily located in one unit, many duties and assignments overlap.

The last year has seen major growth in the size and scope of the research program, coincident with the creation of the separate branch. The major growth areas have been as follows:

1. The neurochemistry laboratory: although space and personnel limitations have been real factors, the rather remarkable growth of the laboratory program reflects Dr. Marangos' success in establishing fruitful collaborations with a variety of other laboratories within our institute and other institutes.
2. The outpatient program has grown substantially, and will continue to grow as resources permit, in anticipation of the ambulatory care facility. The substantial increase in the number of patients treated has been made possible by the assignment of Dr. Gold to the outpatient department as his major clinical and administrative responsibility, by the assignment of Dr. Zis (a visiting scientist) to the outpatient department, by shifting some of Mrs. Davenport's clinical duties from the inpatient unit to the outpatient unit, and by the substantial contribution of Ms. Gay Grover, assigned by the psychiatric nursing service to the outpatient unit.
3. Study of the activity-rest cycle in manic-depressive illness and in normals has expanded. Dr. Wehr has become the only clinical investigator in the Intramural Research Program whose major research focus is on activity studies. A close working relationship between Dr. Wehr and the Technical Development Section (Ted Colburn) has developed, allowing Dr. Wehr to participate in the ongoing development and improvement of the activity monitors and the data collection systems. The formation of a unit on activity studies is being proposed in order to coordinate a wide variety of studies and to standardize methodological and analytical techniques.

4. The development by Drs. Lewy and Markey of a highly specific and sensitive gas chromatographic-mass spectroscopic assay for melatonin represents a breakthrough; the initiation by Dr. Lewy of intraventricular studies in monkeys (in collaboration with Dr. Ebert) is an important related development.

5. The neuroendocrine studies under Dr. Gold have expanded rapidly. Here too the growth reflects Dr. Gold's success in establishing workable collaborative relationships with other groups, both in and outside the NIH. Many of these collaborations have grown out of Dr. Gold's position as the NIMH representative in the NIH Intramural Endocrine Program.

Clinical studies of patients with affective and schizoaffective illnesses entail the integration of biochemical, pharmacological and behavioral observations. Much of the effort of the Branch must be devoted to ongoing development of methods in these areas and to the evaluation of a variety of sources of variance in the data obtained. In the clinical sphere, the new Research Diagnostic Criteria of Spitzer, et al. are being evaluated. Application of the SADS (Schizophrenia and Affective Disorder Rating System) instrument has been implemented, with the goal of increasing the precision of diagnostic and clinical descriptive subtypings for later correlations of responses with biological and pharmacological variables.

Patients with well-established histories of regular cycles or affective disorders are being studied with particular emphasis on whether the predicted "switch" days can be advanced or retarded by various behavioral modifications such as sleep deprivation, day-night reversal, or maintaining the patient on several consecutive "21-hour days." The evidence suggests that a series of 21-hour days can result in a change in cycle length in the predicted direction.

In collaboration with Drs. Markey and Zavadil, we have continued our studies of levels of imipramine (IMI) and desmethylinipramine (DMI) in the plasma and CSF of patients receiving IMI as an antidepressant. There is a very high correlation between the level of both drugs in plasma and in CSF, but the ratio of DMI to IMI is higher in the CSF than in the plasma; this has important implications since the effects of IMI administration on the serotonin metabolite in CSF shows a positive correlation with IMI levels but no correlation with DMI levels. Clinical response to imipramine correlates with levels of IMI, but not DMI, in plasma and CSF. The affinity of ³H-imipramine for brain tissue has been shown to be 45 times greater than for plasma protein, a finding which probably renders meaningless the studies of plasma protein "binding" by equilibrium dialysis against buffer.

Studies of metabolites of amines in CSF utilize probenecid to block transport of acid metabolites from the CSF to blood. Continued collection of data on drug-free depressed patients has resulted in a total sample size large enough to examine the possibilities of the existence of subgroups. A non-normal distribution is evident for the rate of probenecid-induced accumulation of the serotonin metabolite (5HIAA); however, much of the non-normal distribution is related to sex differences in 5HIAA. Responders to

imipramine show significantly higher pretreatment 5HIAA as compared to non-responders. Probenecid levels in CSF have been found to correlate with metabolite accumulation but the extent of this correlation is most pronounced in the bipolar patient group.

In collaboration with the National Naval Medical Center, a study of CSF amine metabolites in alcoholics in the withdrawal phase and on disulfiram treatment has been completed; findings indicate increased levels of 5HIAA during the acute intoxication withdrawal phase followed by a decrease in the abstinence phase to levels below that of controls.

Urinary 3-methoxy-4-hydroxyphenylglycol (MHPG), a norepinephrine metabolite, is influenced by a variety of factors such as stress, diet, and physical activity in depressed patients, but not in normal controls. This observation raises an important methodological question concerning the usefulness of normal subjects as controls for the contribution of these variables to amine metabolite changes in depressed patients and suggests that defective "buffering" systems for the regulation of norepinephrine turnover should be considered in affective illness. There is a clear diurnal pattern for urinary MHPG excretion within normal controls, and in depressed patients, but the timing of the pattern is significantly different in the two groups. Longitudinal studies of urinary MHPG in cyclic manic-depressive patients indicate that, in the same patient, greater amounts of this metabolite are excreted in the manic phases than in the depressive phases. However, it appears that the urinary MHPG alterations follow an erratic pattern of fluctuation which is somewhat out of phase with the mood cycle. To examine possible cause-effect relationships, this data is now being subjected to time-series analysis, including complex demodulation.

Previous work done in collaboration with Dr. Murphy, showed that L-tryptophan (at doses up to 9 gms a day) produces little or no antidepressant or antimanic effects. When this amino acid precursor of serotonin is administered following pretreatment with lithium, however, behavioral effects become apparent. Preliminary data suggest that, used in this way, tryptophan has some antidepressant effects, and in high doses may have psychotogenic effects, particularly in individuals who are already manic.

A serotonin receptor blocker, cyproheptadine, is being tried as an antidepressant; there is evidence that this drug can decrease the hyperactivity of the hypothalamic-pituitary-adrenal axis (HPA) in Cushing's disease (decrease the positive serotonin drive to the HPA) and that this axis may be hyperactive in some forms of depressive illness, particularly unipolar depression with high anxiety and/or psychosis. Results are as yet inconclusive, although one of the two patients treated with the drug to date appeared to have a clearcut antidepressant response.

In collaboration with Dr. Post, the efficacy of pimozide in the treatment of manic psychosis is under investigation. This drug is being compared with chlorpromazine as a reference neuroleptic. Some manic patients have responded dramatically, but others have shown only a gradual and incomplete improvement. Enhanced probenecid-induced accumulation of HVA in CSF of

patients on pimozide is not as consistent as is seen in patients treated with chlorpromazine.

Longitudinal studies of behavioral, physiological and biochemical variables in patients with affective illness are being conducted by Dr. Thomas Wehr. Some patients with regularly cycling bipolar affective illnesses show circadian desynchronization in temperature so that the normal rhythm is no longer "locked in" to the environmental day/night cycle, but tends to drift in and out of phase. Alteration in this normal cycle has not only been found to occur spontaneously in some patients, but can be manipulated by such procedures as day/night reversal and artificial 21-hour days.

Studies of the activity-rest cycle: In collaboration with the Section on Technical Development a new ambulatory non-telemetric physiological monitor has undergone continued development and testing in both inpatients and outpatients. Using these monitors clear and significant differences in activity between manic and depressed patients can be demonstrated and some sleep parameters can be monitored. The relationship between biological rhythms and various neuroendocrine parameters in cyclic manic-depressive illness has also been explored.

A group of neuroendocrine studies have been undertaken by Dr. Philip Gold. Arginine, TRF, and LRF are administered simultaneously and blood levels of TSH, prolactin, growth hormone, FSH, and LH are examined. Since each of these systems are regulated by aminergic pathways which may be different but overlap, the integrated evaluation may allow for a more precise "window" into brain amine systems. Previous reports of a blunted TSH response to TRH in depressed patients have been confirmed and a significant difference between unipolar (lower) and bipolar (higher) patients has been found. The TSH response to TRH is negatively correlated with probenecid-induced accumulation of 5HIAA in CSF. The growth hormone response to L-DOPA is significantly lower in unipolar compared to bipolar depressed patients.

Studies of the response of urinary excretion of free cortisol (UFC) have confirmed that some depressed patients fail to suppress cortisol excretion in response to dexamethasone. Baseline UFC levels are significantly elevated in unipolar patients with psychosis and among unipolar patients the UFC measure correlates positively with CSF 5HIAA.

Outpatient studies have been expanded to examine and followup patients in a "well state" during maintenance on lithium combined with psychotherapy, and for comparison of the efficacy of lithium versus lithium plus imipramine in preventing recurrence of affective illness.

Under the direction of Dr. Paul Marangos, studies have been initiated on brain proteins and polypeptides and how they are altered by psychoactive drugs. Dr. Marangos has continued work (started at the Roche Institute) on neuron specific protein (NSP) and has characterized it as having enolase properties. A second enolase has been characterized and found to be localized exclusively in glial cells. Studies of neuroblastoma cells in culture

(with Eberhard Trams) support the notion that NSP is a molecular marker of differentiation in these cells. The functional significance of the neuronal and non-neuronal form of brain enolase is being explored in relation to brain energy metabolism. In addition, antibodies to a number of brain specific proteins (enzymes and receptors) are being prepared for use in immunohistochemical localization studies.

Dr. Alfred Lewy, working in Dr. Sandy Markey's lab, has developed a gas chromatographic-mass spectroscopic (GC-MS) assay for melatonin that is far more specific and sensitive than any previously available assay. In addition, an immunoassay has been developed and its specificity is being evaluated by comparison with the GC-MS method. The sensitivity of the GC-MS assay (less than 1 picogram) will make possible the detailed investigation of the diurnal pattern of this important neurohumoral substance.

Annual Report of the Laboratory of Clinical Science
National Institute of Mental Health
Irwin J. Kopin, M.D., Chief
July 1, 1976 through September 30, 1977

Introduction

The research programs of the Laboratory of Clinical Science range in scope from studies directed at elucidating fundamental molecular mechanisms of neuronal activity to clinical investigations of the biological bases for neuropsychiatric disorders and for rational pharmacological treatments of these disorders. During this year, two of the six Sections of the Laboratory (Section on Psychiatry and Section on Clinical Neuropharmacology) have themselves become separate laboratories so that there remain a total of four Sections (Experimental Therapeutics, Histopharmacology, Medicine, and Pharmacology). In the Office of the Chief there are two Units (Clinical Biochemistry and Pharmacological Applications of Mass Spectroscopy) which carry out independent research on development of methods useful in clinical studies. These Units play a major role in support of clinical studies in the Laboratory of Clinical Science and in other, particularly clinical, research carried out in other branches of the Intramural Research Program.

The programs of research are directed at understanding fundamental processes which are important for function of the nervous system, at determining how genetic factors, hormones, drugs, or environmental conditions alter these processes, and at explaining their role in various disease states and how they may be modified by therapeutic procedures. These objectives are attained in the several sections of the laboratory through independent lines of investigation at various levels of biological complexity combined with ample opportunity for rapid and efficient application of new fundamental concepts, biochemical assays, and isotopic procedures to clinical studies. There is also stimulation of basic research by questions raised in clinical investigations. The common interests and cross-fertilization of the clinical and fundamental research programs is not limited by the administrative divisions of the laboratory and there is considerable collaboration between the various independent units and sections within the laboratory as well as with other laboratories in NIMH or in other institutes of NIH.

Section on Pharmacology Julius Axelrod, Ph.D., Chief

The investigations in the Section on Pharmacology continues to examine the mechanisms of release of neurotransmitters, the interactions of neurotransmitters with receptors, and the role of neurotransmitters in neuroendocrine, cardiovascular, and brain

function as well as the biochemical processes for synthesis and metabolism of neurotransmitters.

A sensitive method for determination of catechol estrogens (a major metabolite of estrogen) has been developed (Drs. Paul and Axelrod), making possible measurement of tissue levels of this physiologically active compound. Catechol estrogens have been found in the brain and the pituitary gland, as well as in the ovary. The enzyme that converts estrogens to catechol estrogen has been found in brain. It is localized in microsomes and requires P₄₅₀ (the first such enzyme described in the brain). Catechol estrogen has been shown to block estrogen-induced stimulation of formation cAMP in the brain (Drs. Paul and Skolnick).

The subcellular localization of protein carboxymethylase in various lobes of the pituitary has been described (Drs. Gagnon and Axelrod). Protein carboxymethylase is present mainly in the cytosol of the three pituitary lobes (posterior, anterior and intermediate) and in the vesicles of the anterior lobe. Acceptor proteins for the enzyme are highly localized in the intravesicular fraction of all three pituitary lobes. When neurophysin II binds to oxytocin the conformation of neurophysin is changed so that it is a more effective substrate for protein carboxymethylation (Drs. Diliberto and Chaiken). Protein carboxymethylation and its acceptor proteins have been found to be present in red cells and platelets. Thrombin stimulates protein carboxymethylation in platelets (Drs. O'Dea, Viveros, and Axelrod). There is a rapid, transient, elevation of protein carboxymethylation when chemotaxis in leukocytes is stimulated by a bacterial peptide (Drs. O'Dea, Viveros, and Schiffman). A clear relationship between leukocyte chemotaxis and protein carboxylation has been established, indicating that such methylation is an important event in phagocytosis.

Previously we have found elevated levels of phenylethanolamine-N-methyl transferase (PNMT) in the brain stem in 4-week old spontaneous hypertensive (SHR) rats (Drs. Saavedra, Grobecker, and Axelrod). In adult SHR rats, when blood pressure is fully elevated, PNMT in the brain stem returns to normal values. There is a decrease in levels of noradrenaline and of dopamine- β -hydroxylase in selective areas of the hypothalamus in young and adult SHR rats and in rats made hypertensive by administration of desoxycorticosterone (DOCA) and salt. Immobilization stress of rats causes a fall in adrenaline and noradrenaline in areas of the anterior hypothalamus physiologically connected to the function of the adrenal medulla (Drs. Saavedra and Kvetnansky).

Catecholamines have been shown to be present in individual raphe nuclei of the rat (Drs. Saavedra and Grobecker). Inhibition of catecholamine synthesis results in changes in serotonin

metabolism in selective raphe nuclei. These results indicate a relationship between catecholamine neurons and certain serotonergic cell bodies. Destruction of dopamine cell bodies in A₉ and A₁₀ regions of the brain by administration of 6-hydroxydopamine selectively increased glutamic acid decarboxylase in the ipsilateral caudate nucleus and substantia nigra indicating a relationship between GABAergic and dopaminergic pathways (Drs. Saavedra and Keibian). Octopamine is present in human platelets and it is increased many-fold after the chronic administration of monoamine oxidase inhibitors to depressed patients (Drs. Saavedra, Murphy, and Settler).

Phenylethylamine is present in neurons of Aplysia (Drs. Saavedra and Ribas). Ionophoretic applications of phenylethylamine to single Aplysia cells results in an electric response and changes in membrane permeability suggesting that phenylethylamine receptors might be present.

There is a marked circadian rhythm in the post junctional sensitivity of the pineal cells to stimulation by β -adrenergic agonists (Drs. Zatz and O'Dea). Multiple sites are involved in these changes in sensitivity; alterations in β -adrenergic binding sites on cell membrane, adenylate cyclase and cyclic AMP-dependent protein kinase.

Unlike cyclic AMP, which is mainly related to postsynaptic events, cyclic GMP appears to be involved in presynaptic events (Drs. Zatz and O'Dea). Upon stimulation of the pineal, cyclic GMP is released from nerves and cyclic AMP is released from postsynaptic cells. Cyclic GMP is also liberated from peptidergic nerve endings of the posterior pituitary. The release of cyclic GMP is dependent on Ca⁺⁺ and is related to the exocytotic release of the neurotransmitter.

The two neurophysin 'carrier' proteins which are made by cells in the supraoptic nucleus of the hypothalamus are synthesized as parts of larger precursor molecules (Drs. Brownstein and Gainer). These precursors are first processed into smaller, intermediate-sized proteins, and the intermediates give rise to the neurophysins. The two hormones of the posterior pituitary--vasopressin and oxytocin--are likely to be synthesized as parts of the neurophysin precursor proteins.

Thyrotropin releasing hormone (TRH) is present in high concentrations in the hypothalamus (Dr. Brownstein). It can be released from hypothalamic synaptosomes by depolarization with K⁺ in the presence, but not in the absence, of Ca⁺⁺ (Drs. Schaeffer, Brownstein, and Axelrod). It is also released from hypothalamic synaptosomes by dopamine and this action is blocked by antipsychotic drugs.

TRH is present in a clone of cells derived from retina (Drs. Schaeffer, Brownstein, and Shain). TRH is also found in the retina itself. The level of TRH increases when animals are exposed to light and decreases when they are placed in the dark (Drs. Schaeffer, Brownstein, and Axelrod). Thus, TRH may play an important role in vision.

ACTH has been shown to be present throughout the brain and probably has an important action in the CNS distinct from its role in the periphery (Drs. Brownstein and Krieger). It is found in particularly high concentrations in limbic structures where its levels are not altered by hypophysectomy. β -lipotropin and β -endorphin were found in brains of cows and sheep. The distributions of these two proteins were fairly similar. On the other hand, ACTH (which seems to be synthesized from the same precursor protein as β -lipotropin and β -endorphin) has a somewhat different regional distribution. This hints at the importance of peptidase activity in determining a cell's hormonal character.

The regional distribution of neurotensin has been determined, and the effects of diverse lesions on the levels of neurotensin, substance P, TRH, somatostatin, and glutamic acid decarboxylase have been studied as well (Drs. Brownstein and Leeman).

Section on Histopharmacology
David M. Jacobowitz, Ph.D., Chief

This section carries out histochemical and neurochemical analyses of discrete brain nuclei and neuronal pathways in order to define the underlying bases for central neurotransmitter involvement in behavioral regulation.

Histochemical and neurochemical studies are used to map central aminergic, cholinergic, and GABAergic pathways. Stereotaxic maps of noradrenergic pathways in the rat brain have been prepared. A complete atlas of monkey brain showing the localization of catecholaminergic neurons (cell bodies and nerve terminals) and serotonin-containing cell bodies has been prepared (with Dr. Paul MacLean).

The ascending projection areas of the A5 noradrenergic cell bodies of the rat brain was mapped (Drs. Crowley, Speciale, and Mr. O'Donohue) by microdissection of specific brain nuclei and catecholamine analysis after discrete unilateral lesions of the A5 noradrenergic cell group. The lesions resulted in a reduction of the norepinephrine concentration in the medial preoptic nucleus, anterior hypothalamus, median eminence, central amygdaloid nucleus, piriform cortex, bed nucleus of the stria terminalis and caudate nucleus. Surprisingly, a reduction in dopamine concentration was observed in the nucleus accumbens, caudate nucleus, and the median eminence. These changes in dopamine suggest a noradrenergic-dopaminergic interaction.

Intraventricular injection of the antibody to dopamine- β -hydroxylase (ADBH) resulted in selective uptake of the antibody in noradrenergic nerve terminals and its transport by retrograde flow mainly to the locus coeruleus (Dr. Silver). Some adrenergic cells that contribute axons to the ventral noradrenergic pathway were observed to contain the ADBH. Bilateral transection of the dorsal noradrenergic bundle, which is derived from the locus coeruleus, prevented the retrograde flow to the locus coeruleus. Injection of the ADBH into the dorsal bundle - median forebrain bundle resulted in the appearance of the antibody-DBH complex into locus coeruleus and ventral bundle cell bodies (A1, A2, A5). This method is of value in the elucidation of specific noradrenergic pathways which are not seen with previously used histochemical procedures.

Evidence has been obtained which suggests that the stria medullaris (SM) is a major fiber system which connects fore- and hindbrain and contains GABAergic afferents to the lateral habenula (Dr. Gottesfeld). Following SM lesions, it was found that in the lateral habenula glutamic acid decarboxylase (GAD) activity was decreased by 57% and the uptake of GABA by synaptosomes was reduced by 62%. The medial habenula was not affected. The lateral and medial habenula manifest a differential uptake capacity for GABA and glutamate; there is a higher accumulation of these amino acids by the lateral habenula. GABA uptake is predominantly by synaptosomes rather than glia, as shown by drug studies. Part of the GABAergic projection to the lateral habenula appears to originate from the globus pallidus and interpeduncular nuclei.

By employing a microdissection technique and sensitive radio-metric assays, the distributions of acetylcholine (ACh), choline acetyltransferase (CAT), and acetylcholinesterase (AChE) were examined in 60 discrete areas of rat forebrain (Dr. Hoover and Mr. Muth). A 5-fold range was observed for ACh levels, while that for the cholinergic enzymes was about 100-fold. AChE activity was always higher than that of CAT. ACh levels did not appear to be related to the activity of either enzyme in a simple manner. However, there was a good overall correlation between CAT and AChE activities. Such biochemical measurements should prove valuable in future experiments designed to determine cholinergic function and to localize cholinergic pathways.

A recent undertaking in this laboratory is concerned with the neurophysiology of sexual behavior and gonadotropin secretion. The following studies indicate the various approaches to these problems. The effects of prenatal stress on catecholamine concentrations in 24 discrete brain areas of rat offspring, when they became adults, were studied (Drs. Moyer and Herrenkohl). When adults, prenatally-stressed male offspring had significantly lower norepinephrine concentrations in the medial preoptic

nucleus and median eminence. Prenatally-stressed female offspring had significantly higher norepinephrine concentrations in the entorhinal cortex, higher dopamine concentrations in the arcuate nucleus, and lower dopamine in the nucleus accumbens and periventricular nucleus than did normal adult females. These findings correlate with prior findings that prenatal stress feminizes sexual behavior of adult male offspring and suggest that brain neurochemical alterations may play a role in the behavioral changes. This study also examined the effects of stress during pregnancy on catecholamine concentrations in discrete brain areas of the rat. Prepartum stress selectively reduced NE in the medial preoptic nucleus, the anterior hypothalamus, the median forebrain bundle. In the median eminence, DA was reduced significantly. Prepartum stress selectively increased NE in the nucleus accumbens and DA in the nucleus amygdaloideus corticalis.

A study was undertaken to determine whether sex differences exist in catecholamine concentration in discrete brain nuclei and whether such differences could be affected by manipulating neonatal androgen (Dr. Crowley and Mr. O'Donohue). Male rats had higher levels of NE than did females in the preoptic, supra-chiasmatic, arcuate, periventricular, paraventricular nuclei, and in the median eminence. These differences were not affected by either castration of males on day 1 of life or by administration of testosterone to females on day 4 of life. Males had higher levels of DA than females in the arcuate, caudate, median eminence and diagonal band nuclei. Neonatal castration of males lowered dopamine in the arcuate and diagonal band, and neonatal administration of testosterone to females elevated dopamine in these nuclei. These studies indicate that catecholamines may be permanently altered in some areas during the androgen-dependent processes of sexual differentiation, and these changes may underlie, in part, the sex differences in behavior, gonadotropin secretion, and sensitivity to hormones.

Levels of oxytocin and vasopressin in the pituitary during the estrous cycle were determined (Drs. Crowley, George, and Mr. O'Donohue). Pituitary contents of oxytocin and vasopressin were highest at proestrus and estrus, and lowest at metestrus and diestrus. Norepinephrine levels in the paraventricular nucleus were lowest during proestrus and estrus, and highest during metestrus and diestrus, but did not change in the supraoptic nucleus. This demonstrates a relationship between reproductive state and levels of posterior pituitary hormones and suggests a role for oxytocin and vasopressin during the estrous cycle.

A study to determine whether catecholamine concentrations change in discrete brain nuclei during the estrous cycle revealed that NE decreased in the medial preoptic and paraventricular nuclei on proestrus and increased on metestrus (Dr. Crowley and Mr. O'Donohue). NE increased on metestrus in the lateral septum

and central grey catecholamine area. Dopamine decreased on proestrus in the caudate and rose on diestrus. Dopamine rose on diestrus in the lateral septum and on metestrus in the nucleus of the diagonal band. Dopamine increased from proestrus to estrus in the medium eminence. This study helps to delineate how ovarian hormones interact with central neurotransmitter systems to regulate gonadotropin secretion, ovulation, and mating behavior and the neuroanatomical loci for this control.

Section on Experimental Therapeutics
Michael H. Ebert, M.D., Chief

During the past year, the Section on Experimental Therapeutics has focused on a variety of disorders involving the central nervous system and has supported several clinical pharmacological studies. The clinical disorders studied have included Huntington's chorea, tardive dyskinesia, hyperactive syndrome of childhood, Gilles de la Tourette's disease, orthostatic hypotension of various etiologies, anorexia nervosa, narcolepsy, and a drug-induced parkinsonian syndrome in a single patient.

The section has continued to pursue a series of related projects on clinical approaches to the measurement of central nervous system amine metabolism in collaboration with Dr. Kopin and Drs. Kartzinell and Perlow of NINCDS. The rationale of these experiments is to develop more accurate and quantitative clinical techniques which can be used multiple times without hazard or significant discomfort in normal controls as well as patients. Those experiments involving cerebrospinal fluid (CSF) chemistry are first carried out in rhesus macaque primates. Three such experiments are discussed below.

The effects of blockade of transport of acidic CSF amine metabolites by intravenous administration of 40, 80, 160 mg/kg of probenecid on homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5HIAA) in lateral ventricle and lumbar space of the rhesus macaque has been examined (Drs. Ebert and Kartzinell). In clinical metabolic studies, 40 mg/kg is administered intravenously but in rats, 160 mg/kg is required to produce complete blockade of transport. In monkeys, maximum concentrations of probenecid in ventricular CSF were attained 2 hours earlier, and were 40% higher, than in lumbar CSF. The apparent half-life of probenecid in plasma is significantly increased with the increased doses of probenecid. The probenecid-induced rise in HVA and 5HIAA levels in lumbar CSF is significantly greater with increasing doses of probenecid, but this effect is not seen in ventricular CSF. Significant positive correlations were found between CSF levels of probenecid, HVA, and 5HIAA in both ventricular and lumbar CSF.

Stable isotopes have been used to examine the rate of synthesis of the major dopamine metabolite, HVA, appearing in brain and CSF (Drs. Ebert, Kopin, and Perlow). The kinetics of the isotopic species can be used to estimate turnover rates. Methionine, labelled with deuterium on the methyl group (Me-CD₃), administered intravenously to rats, results in a progressive increase in HVA-CD₃ which can be used to measure the rate of HVA formation. In primates, Me-CD₃ administered intravenously appears in lateral ventricular CSF. The relative enrichment of Me with CD₃ progressively decreases while that of HVA increases until it reaches that of the Me. After the relative enrichment of HVA reaches this peak, it declines more slowly than that of the Me. This precursor-product relationship can be used to estimate the turnover constant for removal of HVA from CSF. In similar experiments using ¹⁸O to label dopamine, the kinetics of ¹⁸O-HVA appearance and disappearance in CSF can be used to estimate dopamine turnover.

Changes in plasma levels of deuterium-labelled HVA (d₂-HVA) administered intravenously can be used to determine turnover rates and volume of distribution of HVA in patients (Drs. Kopin, Ebert, Gordon, and Zavadil). HVA disappears from the plasma with an apparent half-life of approximately 40 minutes (K_E = 1.05). Synthesis rates for HVA calculated from plasma levels of HVA, the K_E of d₂-HVA and the volume of distribution d₂-HVA agree favorably with urinary excretion rates of HVA. After 75 minutes, an infusion of probenecid (40 mg/kg), is administered to block transport of the acid. The excretion rate of d₂-HVA decreases by 70-90% and levels of endogenous HVA in plasma increase. Calculations of the rate of entry of HVA into plasma before and after probenecid reveals entry of HVA from a probenecid sensitive HVA compartment (presumably this is primarily the central nervous system). It appears that about 50% of HVA produced in the body originates from this source.

The clinical programs include studies of the pharmacological treatment of the hyperactive syndrome of childhood, physiological and pharmacological studies of anorexia nervosa, and studies of neuropsychiatric disorders, particularly Huntington's chorea and Gilles de la Tourette's syndrome. The studies of the hyperactive syndrome of childhood are pursued in collaboration with the Biological Psychiatry Branch and parts of the study are also discussed in their annual report. Studies on the effects and metabolism of a single dose of d-amphetamine administered to hyperactive children were concluded this year. Plasma levels of amphetamine reach a peak of approximately 60 ng/ml between 3-4 hours after a single oral dose of 0.5 mg/kg, and subsequently fall with an apparent half-life of 6 hours. There is a significant response in behavioral pathology, but not in motor activity, to a single oral dose of amphetamine when compared with a placebo. Behavioral response is maximal between 2-3 hours after drug administration while plasma amphetamine level is rising rapidly.

Studies of cortical average evoked response in relation to plasma amphetamine level and behavioral response are in progress (Dr. Buchsbaum). Initial data analysis in these studies demonstrates a highly significant correlation between a decrease in variance of average evoked response and plasma amphetamine level at 2 and 4 hours after administration of the drug. Studies of psycholinguistic parameters (Dr. Ludlow, NINCDS) show that hyperactive children treated with amphetamine (compared to placebo) show an increase in language comprehension, speak more slowly, produce fewer echolalic and stereotypic responses, and produce relatively less speech not relevant to examiner requests. Patients with Huntington's Disease (Drs. Caine and Weingartner, LPP) are unable to utilize normally significant aids (e.g., high verbal imagery) for encoding information deeply enough to allow consistent retrieval. Patients fail also to process new items at a normal rate of speed. In collaboration with Dr. Evarts (LNP) and Dr. Ludlow (NINCDS) psychophysiological studies are being used to investigate the ability of Huntington's patients to utilize kinesthetic and auditory stimuli. A pilot study of pharmacological effects on memory function is in progress. The effect of acute cholinergic and dopaminergic stimulation and blockade on various parameters of memory function in Huntington's patients and normal controls is being studied.

In studies of thirty patients with Gilles de la Tourette's syndrome (Dr. Caine), eleven of whom were admitted to the hospital for intensive study, acute, provocative pharmacological testing with d-amphetamine, l-amphetamine, and haloperidol suggests that the pathophysiology of the disorder may be biochemically heterogeneous. Linguistic studies (with Dr. Ludlow) demonstrate language and speech deficits in most patients; the former is highly correlated with an increased variety of vocalization. The problem of tardive dyskinesia in Tourette's patients, who are usually treated with haloperidol, has become a focus for developing new therapeutic agents. Clozapine, a non-neuroleptic antipsychotic, is being studied intensively in Tourette's patients. Drug trials using clozapine are in progress in patients with tardive dyskinesia or Huntington's Disease. This agent may have considerable clinical significance because it is believed to be effective without causing tardive dyskinesia.

Abnormalities in prolactin regulation have been found in patients with Huntington's Chorea. Baseline plasma prolactin levels are abnormally high compared to healthy controls and the patients fail to respond normally to the suppressive effects of l-dopa and bromocryptine. Although they have increased prolactin release after chlorpromazine, their maximum prolactin levels are significantly lower than controls. These results suggest that Huntington's patients have deteriorated dopamine-mediated control mechanisms for prolactin release.

A clinical research program in anorexia nervosa has been fully developed this year by Dr. Gross. Patients who are acutely ill are being admitted for study in a program which includes a behavior modification regimen as a baseline treatment. An initial evaluation period includes several studies of central and peripheral monoamine metabolism. Several drug trials of agents known to stimulate appetite are underway. Preliminary results of a trial of lithium carbonate in promoting weight gain in acute anorexia nervosa suggest that it is efficacious. A clinical trial of Δ -9-tetrahydrocannabinol has been approved by the FDA and will begin soon. A study of gastric function in anorexia nervosa with Dr. Dubois (National Naval Medical Center) has demonstrated that during the acute phase, fractional emptying rate is decreased two-fold, and intragastric volume is increased two-fold. A study of cardiac function during the acute phase [with Drs. Gottdiener, Henry, and Borer (NHLBI)] indicates that although left ventricular mechanical function is intact, ventricular extrasystoles (VAC's) occur frequently and in clear association with decreased left ventricular diastolic dimension and wall thickness. Data collection on psychological characteristics are hoped to predict favorable response to behavior modification or drug treatment.

Section on Medicine
Irwin J. Kopin, M.D., Chief

Methods for obtaining blood from awake, undisturbed rats (Drs. Popper and Chiueh) and for sensitive, specific radio-enzymatic assay of plasma catecholamines (Mrs. Weise and Dr. Chiueh) have been applied to studies of the effects of drugs, environmental manipulations, genetic differences, and brain lesions or stimulation on plasma catecholamine levels. Handling, exposure to cold, or mild stresses produce significant increases in plasma catecholamine levels (Drs. Popper, Chiueh, and McCarty). The levels are increased markedly during immobilization or after administration of 2-desoxyglucose (2DG), which mimics hypoglycemia (Drs. Sun and Thoa). These responses, and those of corticosterone, are markedly attenuated by hypothalamic differentiation. Catecholamine responses, but not corticosterone increases, after 2DG are partially blocked by severing posterior hypothalamic tracts (Drs. Sun and Thoa). Decapitation produces extremely marked increases in plasma catecholamines within several seconds, presumably as a result of massive sympathetic discharge attending 'spinal shock.' The increases in epinephrine are even more striking than those of norepinephrine. Anesthetic agents of various types almost completely block the spinal shock-induced increase in plasma catecholamines attending decapitation (Drs. Rosin, Moss, Henry, and Weise) but in rats previously immobilized (Dr. Kvetnansky) or in mice exposed to attack (Drs. Tizabi, Maengwyn-Davies, and Thoa) there is an enhancement of the increases in plasma catecholamines produced by decapitation. The enhanced response to decapitation of immobilized rats is found in adrenalectomized,

as well as in intact rats, indicating that it is independent of adrenal cortical hormones.

Spontaneously hypertensive (SHR) rats are hyperresponsive to restraint in containers used for measurement of blood pressure (Dr. Chiueh), and to immobilization stress as well as decapitation, but not to exposure to cold. Both behavioral responses and increases in plasma levels of catecholamines and corticosteroids and blood pressure and heart rate are greater in SHR than normotensive (WKY) control animals (Drs. McCarty and Chiueh). There also appears to be an abnormality in afferent or central cholinergic mechanisms involved in control of adrenergic responses in SHR rats (Drs. McCarty and Weinstock).

Plasma catecholamines are elevated by chemical stimulation of central cholinergic receptors by administration of oxytremorine. The drug causes tremor by a central cholinergic mechanism and the tremor is enhanced by the attendant increase in peripheral catecholamine secretion (Drs. Weinstock, Zavadi, Rosin, and Chiueh). Blockade of β -adrenergic receptors, destruction of the sympathetic nerves by pretreatment with 6-hydroxydopamine, or adrenal medullectomy attenuates the tremor, but not peripheral cholinergic responses.

Administration of cocaine results in a centrally-mediated increase in plasma catecholamines. The increase in epinephrine greatly exceeds that of norepinephrine and becomes most evident when catechol-O-methyl transferase is inhibited. The increased levels of epinephrine are not the result of blockade of uptake because it is not found when other drugs, such as desmethlimipramine, which inhibit amine uptake are administered and the response is attenuated by adrenal denervation.

Plasma catecholamines can be increased by stimulation of anterior or posterior areas of the hypothalamus (Drs. Sun and Thoa) and pharmacological studies of this response are in progress. Changes in levels of neurotransmitters and their biosynthetic enzymes in specific areas of brain are indices of involvement of the areas in physiological or pharmacological responses. Anesthetic agents, (Drs. Roizen, Thoa, Zivin and Jacobowitz) elevate levels of serotonin and catecholamines in the amygdala, substantia nigra, and nucleus centralis superior, while immobilization stress appears to have the opposite effect in these and other nuclei (Drs. Palkovits, Brownstein, Kizer, and Saavedra). Glutamic acid decarboxylase and the transmitter, GABA, formed by the action of this enzyme are also involved in central control of the sympathetic nervous system (Dr. Tappaz).

Levels of phenylethanolamine-N-methyl transferase, (PNMT), in the superior cervical ganglia (SCG) of newborn rats are increased by administration of Nerve Growth Factor (NGF) or

dexamethasone (Liuzzi and Foppen). PNMT is responsible for conversion of norepinephrine to epinephrine (EPI), however, greater increases in EPI are found after administration of dexamethasone than after NGF, although NGF stimulates formation of more PNMT than does dexamethasone. This suggests that different types of different localizations of PNMT are stimulated by NGF and dexamethasone. Studies using deuterated methionine have proven that EPI found in the SCG is not formed via epinine, the N-methylated product of dopamine (Drs. Foppen and Liuzzi). Serotonin levels in the SCG are increased by NGF, but not in catecholamine-containing cells (Drs. Liuzzi, Foppen, Levi-Montalcini, and Saavedra).

Studies of plasma catecholamines and dopamine- β -hydroxylase in human subjects (Drs. Lake and Ziegler) have provided a biochemical means of separating three clinical subtypes of orthostatic hypotension. The disorder may be caused by a deficit in the control by the central nervous system of the sympathetic response, a defect in the sympathetic nerves, or an abnormality in the ability of vascular compartment to respond (lack of constrictor response or low blood volume).

Plasma levels of norepinephrine (NE) increase with age, and age-correction appears to eliminate reported higher levels of NE in patients with essential hypertension. During hypoglycemia plasma levels of catecholamines become elevated but in certain endocrine disorders involving the adrenal cortex there are abnormal responses (Dr. Krieger). Low levels of plasma catecholamines and DBH have been found in patients with Lesch-Nyhan Syndrome (Drs. Lake and Ziegler).

NE has also been found in cerebrospinal fluid (CSF). The levels of NE in CSF appear to be related to those in plasma, although NE from plasma is unable to reach the CSF because of the blood-brain barrier. Probenecid administration, which blocks transport of acidic metabolites, elevates levels of NE in plasma and CSF. The levels of NE in CSF of monkeys varies diurnally (Drs. Ziegler, Lake, and Ebert).

Office of the Chief
Irwin J. Kopin, M.D. Chief

The two units in the Office of the Chief support the research efforts of other sections of the laboratory, particularly the clinical research, which require special techniques on Mass Spectroscopy. The units carry out independent research related to development and utilization of the methods required for this support function.

Unit on Pharmacological Applications of Mass Spectrometry

Dr. Sanford Markey, has designed, built, and tested necessary modifications for negative ion chemical ionization mass spectrometry using a quadrupole mass filter. This instrument displays sensitivity gains of 100-1000 over the conventional positive ion analyzer for electron capturing molecules and is being used for a highly sensitive and specific assay of melatonin in plasma and tissues.

Organic synthesis projects in this unit have resulted in a greatly improved method for production of labeled ($^3\text{H}_2$ or $^2\text{H}_2$) diazomethane via charge-exchange catalysis of diazomethane with labeled water (Dr. Markey). A variety of labeled compounds have been and will be synthesized for use in studies of biogenic amine metabolism - homovanillic acid - d_3 , d_8 ; 4-hydroxy-3-methoxyphenylglycol - d_5 , d_3 , d_2 , $^3\text{H}_2$; melatonin - d_4 , d_7 .

The pharmacology of the dopaminergic drug, bromocryptine, remains under study. Demonstration of the presence of unchanged drug in the CNS is being attempted using ^{82}Br labeled compound (Drs. Colburn and Markey).

The distribution of norepinephrine, serotonin, and gamma-aminobutyric acid has been studied in synaptosomes from normal and reserpinized rats. A shift was observed for norepinephrine and serotonin from mainly vesicular binding from untreated rats to cytoplasm in tissues of rats treated with reserpine. Using this technique, the effects of drugs on the subcellular localization of various compounds may be used to examine mechanisms of storage and release of amines and other neurotransmitters. Hypotonic bursting of synaptosomes releases only a portion (presumably cytoplasmic) of labeled neurotransmitters taken up by synaptosomes. The hypotonic resistant portion of the labeled transmitter is presumed to be retained in the vesicles. The proportion retained in vesicles increases with time but appears to be equally susceptible to release, suggesting an equilibration of cytoplasmic and vesicular amines.

Unit on Clinical Biochemistry

Under the leadership of Mrs. Edna Gordon, the unit develops and applies new procedures for the quantitative analysis of metabolites of biogenic amines and related compounds in human and animal CSF, blood, tissue, and urine. Studies on human biological fluids are made in collaboration with clinical investigators, while studies in animals are undertaken to check the utility of new methods, to clarify the pharmacologic action of drugs used clinically, or by sections involved in fundamental research to take advantage of the specialized expertise of the unit.

The major metabolite of norepinephrine (NE) in brain is 3-methoxy-4-hydroxyphenyl glycol (MHPG). Vanillyl mandelic acid (VMA) is also formed but in much smaller quantities. Urine and blood contain VMA and MHPG in three forms - free, conjugated with glucuronic acid (MHPG glucuronide), and as the sulfate (MHPG.SO₄). These compounds are products of NE metabolism in brain and in peripheral tissues and are excreted in the urine. Strategies involving administration of isotopically-labeled compounds to assess the relative amounts of MHPG contributed by brain and periphery require separation and determination of the specific activities of these urinary metabolites. Procedures to separate MHPG, MHPG.SO₄, MHPG. glucuronide, and VMA in human blood and urine are being developed so that the specific activity as well as the absolute amount of each compound can be measured with great accuracy. Deuterated and tritiated MHPG, MHPG.SO₄, and MHPG. glucuronide have been prepared to use as tracers and internal standards. Separation methods using ion exchange and thin-layer chromatography have been worked out. New derivatives are being tested in an attempt to improve the present procedures for the analyses of MHPG by GLC-MS. These procedures are to be applied to studies of NE and dopamine metabolism in human subjects (Drs. Kopin, Ebert, and Gordon).

Annual Report of the Laboratory of Developmental Psychology

National Institute of Mental Health

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Marian Radke-Yarrow, Ph.D., Chief

When the Laboratory was established two and a half years ago, the decision was made to include research on normal and pathological child development and behavior, over the entire age range of childhood. There has been steady progress toward these objectives, with the programmatic profile of the Laboratory becoming well defined in the past year. There are three major areas of ongoing research:

the emotional-social development of children,
child psychopathology, and
the interfaces between organic conditions and child behavior.

The domain of emotional experience and emotional behavior is one of the less well understood and less investigated areas in developmental psychology. Basic research in this area continues in the Laboratory with two general foci. One is on mapping developmental transformations in children's emotional-social behavior; the other is on the experiences of children, especially in the family, in relation to short-term and long-term emotional development. Problems of measurement in this area present difficulties; hence, developments in methodology are given attention in research.

In the area of child psychopathology, the research has continued with a focus on the development of a diagnostic instrument for the reliable and standard classification of childhood behavior disorders. With such an instrument available, evaluation studies of many kinds can be undertaken.

In the Laboratory's research concerned with the interrelations of organic conditions and behavior, there has been a necessary first investment in becoming sophisticated in the particular areas of organic problems in which behavioral research was to be undertaken. As in the preceding problem areas, measurement issues are extremely complex and require new and innovative approaches. Research projects are underway on interrelations of psychological functioning and disease-related and deficiency-related conditions in children.

The three areas of research are in many respects not independent of, and distinct from, one another. The interdependence of problems and methods becomes apparent in the following descriptions of ongoing programs of research and research projects.

Studies of Emotional-Social Development

In the research on early emotional development, Dr. Carolyn Waxler and Dr. Marian Yarrow have focused primarily on empathy and altruism. They have completed a study of 1 to 2½ year-olds in which they have (a) examined the nature of arousal in the child from affective inputs from the environment (i.e., the emotions and moods of others) and (b) identified systematic developmental changes as well as individual differences in children's processing and acting upon such affective input. Children respond to negative feeling states of others well before sophisticated cognitive processing can be assumed. At first, it is a reflex response of global bodily distress; soon developmentally systematic gains in comprehension of others' feeling states can be documented by children's matching of others' expressions, by their acting as if the affect were their own, by their reproducing the mother's caretaking response to the other person's distress, by their direct attempts to intervene sympathetically. These data will help to provide indicators of normal responsivity to feeling states in others, as well as to point up possible pathological signs. The data also point up some of the immediate consequences for young children of affective conditions (e.g., anger between parents, grief, fear) in the family environment.

In a second, also completed, study children's responses to affect were examined in relation to mothers' techniques. Empathy expressed by the child was found to vary positively with maternal behaviors expressive of empathy toward the child and maternal behaviors that increased the child's attending to and discrimination of emotional events. Also, mother's behaviors toward their children influence children's awareness of their own role in causing distress. Certain maternal techniques are associated with early guilt reactions by the child. Data from this study suggest directions for the kinds of parental interventions that can be used to influence affective development in early childhood.

In pursuing the question of cognitive factors in empathic and altruistic behaviors in children 5 to 8 years of age, Dr. David Barrett and Dr. Yarrow found no direct link between children's abilities to make inferences about others' emotional states and children's altruism. However, it was found that a relationship depends in part on personality factors. Children's inferential abilities are positively related to altruism in socially assertive children, but negatively related in nonassertive children. These findings indicate the need for further investigation of the processes involved in altruistic responding.

Investigation of the development of affective dimensions of interpersonal behaviors (nurturing, gentleness, stoicism, assertiveness, emotional expressiveness, etc.) was extended to later ages (5 to 14 years) by Dr. Karabelle Pizzigati and Dr. Charles Halverson. Their major interest was in how similarly or differently boys and girls attribute these dimensions to themselves. Video-taped behaviors were used as stimulus materials. The investigators found that traditionally-viewed feminine traits were incorporated in girls' perceptions of their own affective behaviors with far greater frequency than they were in boys' self perceptions. The sex differences were already present at age five. Boys and girls were equally likely to identify themselves with the traditionally

masculine affective characteristics. Age trends were significant and different for the two sexes. With increasing age, girls change little on self-perceptions in "feminine" terms but increase markedly in describing themselves in "masculine" behaviors. With age, boys increasingly identify themselves with "masculine" behaviors but show sharp decreases in identifying with "feminine" behaviors.

Investigation of the psychological consequences, particularly the emotional consequences, of a major stress in the lives of children is undertaken in a study of children whose mothers have engaged in crime and are imprisoned (Dr. Velma LaPoint and Dr. Yarrow). Lack or loss of stable and supportive parenting is assumed to place children at risk for the development of social and emotional problems. When the reasons for inadequate parenting are themselves indicative of psychological problems of the parent or of stressful environmental conditions, as in these circumstances, risk for the child is compounded. Imprisoned mothers and their children and community-living mothers and their children, from the same neighborhoods, are the research subjects. Children's emotional states, self perceptions, relationships with mother and with authority figures are given attention. From pilot data it was found that children not infrequently are exposed to the illegal and violent actions of their mothers(including murder) in the periods prior to imprisonment. Also, children engage in strong denial and in efforts to conceal their mother's imprisonment. When the mother's status is known, the child is subjected to peer ridicule which in many ways extends the mother's derogated qualities to the child. Data comparing children from the community and the prison families are not yet available. Data from this study should provide insights into factors influencing children's coping with devastating family disruption, and provide bases for recommendations regarding intervention or care for the children of imprisoned parents.

Dr. Jacob Gewirtz's continuing program of research on mother-infant relationships has a two-fold focus: (1) to investigate the influence of the maternal caregiving environment on adaptive, nondistress, and communicative behavior of the child; and (2) to study the influence of the child on its mother and other caregivers. Under the first focus, attention has been directed to ways in which the mother can influence a salient behavior of her infant, nondistress crying, by how she responds to that crying. Under the second focus, attention has been addressed to ways whereby the young infant influences its mother's responding to it, by such behaviors as crying, smiling and vocalizing. In a series of experiments, Dr. Gewirtz and his colleagues have demonstrated how behaviors attributed to 3-month-olds can condition verbal responses and facial expressions of their mothers, without the mothers being aware that they are being influenced. Studies are in progress on how parents' interest in, and attachment to, their infants are influenced by diverse forms of exposure to the infants during the first post-partum hour. The investigations being carried out vary (a) the contact of normally-delivered newborns with their mothers (skin-to-skin contact and cradling) and (b) the duration of interaction of fathers with their Caesarian-section-delivered newborns. The influence of these exposures on the behavioral development of the infants as well as on the parents' attachment is under study. Studies under both foci provide bases for child-rearing recommendations, for the management of infant crying, and for the implementation of post-partum hospital procedures that

foster conditions of parent-infant interchange that are optimal for bonding (i.e., mutual attachment formation between parents and child).

Studies of Child Psychopathology

Dr. Thomas Achenbach's research program has been directed toward the development and testing of a standardized measure of adaptive and maladaptive behaviors in children and adolescents. The primary data collection instrument is the Child Behavior Checklist, through which are obtained parents' descriptions of their children's behavioral competencies and problems. Using checklist data on children in 25 child mental health settings, Dr. Achenbach and Dr. Craig Edelbrock are constructing standardized behavior profiles for children grouped by age and sex. Standardization of profiles for boys aged 6 through 11 and for boys aged 12 through 16 have been completed. Profiles for girls and younger children will follow. For boys, reliable clusters of behavior problems were found in separate factor analyses for each age group (Schizoid, Uncommunicative, Obsessive-Compulsive, Somatic Complaints, Hyperactive, Aggressive and Delinquent). Clusters were found for the younger group indicative of Depression and Social Withdrawal, while for the older group, of Immaturity and Hostile Withdrawal. In the course of the work on standardization of profiles, Dr. Edelbrock has developed some new approaches to cluster analysis.

Behavior profiles make it possible to score individual children on social competencies and behavior problems and to classify children into "types." This provides a means for quantitatively evaluating a child in relation to other variables, e.g., with respect to the child's prognosis over time and differential responsiveness to treatment.

A survey study using the Behavior Checklist has also been conducted with parents of 1300 normal children in order to obtain nonclinical norms.

Further work is continuing to determine the clinical usefulness of the Child Behavior Profiles. Drs. Achenbach and Edelbrock have found that short-term reliabilities are very high; long-term stabilities of profile patterns for treated children are also substantial, despite declines in the number of problems reported; interparent agreement is adequate; and differences between clinic referred and nonreferred (normal) children are highly significant. To broaden the assessment base and to delineate more precisely the relations between profile patterns and treatment outcome, a study is underway of clinic-referred children in which parents, a clinician, and teachers will fill out Child Behavior Checklists. These data will be obtained at intake into a community mental health center and again after termination of treatment in order to determine the degree of agreement among profiles scored from data supplied by the various informants, and to determine how well the profiles predict the outcome of mental health contacts.

This research should aid greatly in meeting a need in research on child psychopathology, namely providing a standardized system for identifying types of childhood disorder.

This is a new direction of research in the Laboratory, and the studies, generally, are in early stages. One group of projects deals with serious illness (cancer) in children. The objectives are to elucidate (a) how illness and treatment affect the cognitive, affective, and social functioning of the child, and (b) how the functioning of familial and medical-care networks is associated with psychological adaptations of the child. Physiological and neurological changes which appear to be a result of treatment are also being related to children's psychosocial status. Dr. Elizabeth Susman and Dr. Albert Hollenbeck are studying the adaptations of children to the extreme of hospitalization, namely isolation in a Laminar Air Flow Unit. This is a protective germ free environment in which children are deprived of direct physical contact with parents, siblings, and peers as well as many sources of stimulation in the environment. These experiences are current with weight loss, alopecia, and painful medical procedures associated with chemotherapeutic agents. Children are randomly assigned to Laminar Air Flow units or a general pediatric oncology ward for a period of five-to-seven weeks while they are receiving chemotherapy.

Attention is given to: (1) how separation influences behaviors normally associated with childhood; (2) the appearance of prolonged periods of anxiety or depression; and (3) the child's and parent's conceptions of the illness. Systematic observation, interviews, and ratings by patient, parent, and nurse are used, with extensive sampling on a continuing basis throughout the period of hospitalization. To date, data have been collected on six research participants. Ten or more children will be studied. Analysis will focus on sequential changes in behavior related to the course of illness and treatment, with particular attention to symptoms of depression. Understanding the impact of illness has implications for the future psychological development of ill children, and has potential generalizability to children's reactions to stress, in general. It is important to identify the specific conditions associated with emotional states, such as depression and withdrawal during illness and recovery, since motivation to engage in age-appropriate interactions may be critical to the well-being of the child and ultimately to survival. Among the social conditions studied are the interactions between child, parent and medical personnel. What kinds of interaction tend to increase anxiety and fear; what kinds are supportive?

In a second study of children with cancer, Dr. Howard Moss is investigating the cognitive characteristics of survivors of leukemia who have undergone intensive central nervous system treatment as a prophylactic measure for sustaining remission. The chance of survival from childhood leukemia has increased greatly over the past ten years because of the utilization of new treatment procedures aimed at destroying stray leukemic cells in the central nervous system. These prophylactic measures consist of a series of cranial irradiations and intrathecal (spinal) injections of powerful chemical agents. Recently, concern has been expressed as to possible deleterious effects on the brain resulting from this treatment. Thirty-six children who have been in remission from acute lymphocytic leukemia for 2 to 5½ years were administered the Wechsler Intelligence Scales and the Graham-Kendall and Bender-Gestalt tests

of perceptual motor functioning. Subjects' performance was compared with that of healthy siblings. The patients' level of functioning on both verbal and nonverbal abilities was significantly lower than that of the siblings. Also, there is some evidence that the more time that has elapsed between CNS treatment and intellectual evaluation, the greater the difference between patients' and siblings' scores. This suggests that CNS treatment may have affected the child's conceptual abilities and capacities to assimilate and integrate new information. Additional data on the children's school-related behavior will be obtained. Also, the degree of deficit in patients will be studied in relation to computerized tomography records obtained by research staff of the Pediatric Oncology Branch.

This study will be repeated with a prospective design. Patients will be evaluated on a test battery prior to CNS treatment and one year after treatment. A subgroup of cases of optimal prognosis who receive intrathecal methatrexate but no CNS irradiation will also be studied prospectively. These studies will be carried out collaboratively with the Hematology Oncology Service of Children's Hospital in Los Angeles.

Several projects are concerned with infant development and early indicators of intellectual dysfunction. Dr. Albert Caron's research has continued on perceptual and cognitive functioning in infants under six months of age. He has investigated the infant's ability to perceive constant shape; i.e., ability to see invariant real shape despite rotation in the third dimension which alters retinal or projective shape. It appears that very young infants perceive the visual cues which specify depth (e.g., motion parallax and binocular disparity cues) but have not yet become able to interpret this optical information in terms of the third dimension. A part of Dr. Caron's program has been devoted to methodological work in assessing infant abilities.

Over the past years Dr. Caron and his colleagues have made many assessments of infant learning and cognition. Since many of these children are now of preschool or early school age, it is possible to investigate the predictive value of the early measures. Infant tests have generally not been predictive of later intellectual functioning. Investigators of infant learning and cognition, however, continue to be impressed with the range of infant differences in performance. To determine the extent of relationship, if any, between infant performance on habituation and learning tasks and later measures of intellectual ability, 100 children were tested on the Stanford Binet at four to seven years. No relation was found between the infant and early childhood measures. Finer analyses will be made of these data, also similar follow-up comparisons will be carried out using discrimination learning as the infant assessment. In another effort to identify early indicators of later intellectual functioning, Dr. Caron is comparing Down's Syndrome infants, who have high probability of intellectual deficiency, with normal infants on a range of habituation and learning tasks.

Dr. Sarah Friedman's research is with high risk infants. Approximately six percent of all births in the United States occur before the 38th week of gestation. Medical technology has made survival possible for infants born prematurely after only 28 weeks of gestation and weighing as little as 800 grams. Although this is an impressive accomplishment, survival is a very gross

measure for evaluating life. It says nothing about the quality of the life of the individual who has survived. Unfortunately, prematurity has been associated with many manifestations of central nervous system dysfunction. Dr. Friedman is asking questions about the ability of prematures at the time of their expected birth to process information which is believed to be an index of the degree of cortical maturity and integrity. Premature infants (well and previously ill) and full-term infants are being tested in three modalities: tactile, auditory and visual. These modalities develop at different rates pre- and postnatally. The tactile modality develops before the auditory and the auditory before the visual. If a sensory system is mature at birth, the additional extrauterine stimulation that a premature receives relative to a full-term newborn may enhance the development of that system. However, if a sensory system is not ready for normal interaction with the extrauterine environment, its development may suffer when the baby is delivered prematurely. Data gathering is now underway.

Another project concerned with relations between physiological conditions and child behavior is a study, in planning stage, of the effects of chronic malnutrition and nutritional supplementation on children's cognitive, social, and emotional development (Dr. Barrett and Dr. Yarrow). Chronic moderate malnutrition is a condition of life for sixty percent of the world's child population. Its most characteristic contexts are the environments of poverty and disease with the inherent hazards and deprivations that they present. For children growing up under these circumstances, there are certain to be many risks to physical and psychological well-being. Much research has been devoted to relations between severe malnutrition in infancy and later intellectual development. More recently there is evidence from experimental intervention studies that consistently low levels of protein intake early in life influence adversely children's intellectual abilities. Very little research attention has been given to the study of relationships between nutritional status and the overall mental health of the child: namely, self-regulatory abilities, emotional or affective qualities, and interpersonal competencies and disorders. The prospective study focuses on these areas. The complex problems of field implementation of this study are currently being worked out, as are the development and modification of instruments suitable for field conditions.

Another study bridging the organic and behavioral is one designed to assess the effects of various hormone abnormalities on behavioral and cognitive patterns (Dr. Edelbrock and Dr. Achenbach). Normal controls and children (ages 6 to 16) who have either thyroid or sex hormone abnormalities are tested before and after hormone treatment on a cognitive battery. The Child Behavior Checklist (described earlier) is also administered. Children with a history of hyperthyroidism show very early closure of the sutures of the skull, and a high proportion of these children have been reported to have school difficulties and problems concentrating and reading. Preliminary findings suggest some unusual patterns of cognitive functioning in children with thyroid abnormalities, but not enough data are yet available to assess relations between cognitive and behavioral patterning with any confidence.

In the next one to two years many of the studies just begun in the past year will have findings to report. In each instance, the data are relevant

to basic knowledge of child development and also have implications for many - issues in the daily lives and welfare of children.

There have been staff changes in the past year, both additions to the Laboratory and departures. Drs. Craig Edelbrock and Albert Hollenbeck came as Staff Fellows. Dr. Elizabeth Susman joined the staff as a Visiting Foreign (Canadian) Fellow. With the retirement of Ms. Frances Polen, the Laboratory has lost a most valued member. Dr. Charles Halverson, a major contributor to the longitudinal study of the Child Research Branch, has left to accept a University appointment.

The Senior Investigators and Staff Fellows have served this year in many capacities in NIMH-NIH and in the discipline as research advisors and consultants and officers of scientific societies and as committee members. Drs. Achenbach and Yarrow serve on advisory committees to Dr. Brown. Drs. Moss and Yarrow are members of the Ethics Review Committee in the Institute of Allergy and Infectious Diseases, Dr. Carolyn Waxler served as Secretary-Treasurer of the Assembly of Scientists. Drs. Caron and Halverson serve on review committees in the intramural program. Drs. Pizzigati and Susman are members of the NIH Day Care Committee. Dr. Pizzigati is a consultant on Day Care to the Office of Assistant Secretary for Planning and Evaluation, DHEW.

The Laboratory is well represented in the major scientific organizations of the discipline: Committee positions are held in the APA and Society for Research in Child Development by Drs. Gewirtz, Halverson, and Yarrow; and in the Social Science Research Council by Dr. Yarrow. Recognition by the discipline is in evidence also by memberships on editorial boards of scientific journals by Drs. Achenbach, Caron, Gewirtz, Moss, Waxler, and Yarrow. Dr. Yarrow has been appointed to the Board of Directors of the Foundations Fund for Research in Psychiatry.

The ongoing research of the Laboratory is carried out not only in the research facilities on the NIH campus; many of the studies are conducted in the field. The research problems dictate the necessary settings. Projects are currently being carried out collaboratively or with the cooperation of community clinics, hospitals, correctional institutions, schools, and families. Our research is greatly aided by the good working relationships with these institutions. We are also able in many instances to provide some feedback from the research activities to the participants in research.

Annual Report of the Laboratory of Neuropsychology

National Institute of Mental Health

H. Enger-Rosvold, Ph.D., Chief

July 1, 1976 - September 30, 1977

This year's annual report summarizes the progress of the Laboratory of Neuropsychology in its first year with Laboratory status. It was not intended that this organizational change would abruptly alter the direction of a research program which had been evolving for twenty-three years. Rather, the thought was to develop a mechanism for increasing the intensity and sophistication with which the disciplines of behavioral neuropharmacology, neuroanatomy, neuroendocrinology, and electrophysiology could be brought to bear on questions arising from neuropsychological studies. Although this plan was never made explicit, existing space and personnel have been gradually distributed so that from a modest multidisciplinary effort in which these disciplines served as adjuncts of neuropsychology, projects evolved which embraced in depth the relevant fundamentals of several of the neuro sciences. In this milieu, productivity this year measured both by quantity and quality, surpassed that of most years. Yet, it is less than could have been achieved had adequate staff been available. Much of the achievement was possible only because guest workers at both the professional and the assistant level could occasionally be encouraged to join us. This arrangement, tenuous at best, does not provide the continuity vital in the long-term studies characteristic of our research.

Discernible in the narrative summary that follows is a best effort report concluding that the research achievement, though considerable, for want of adequate staffing does not approach its potential.

Cortical Mechanisms in Vision

In a collaborative study with Gross at Princeton, we delineated the functional pathway through which inferior temporal (IT) neurons receive their visual information. The method entailed measuring the defects in the visual receptive fields of single IT neurons after selective cerebral lesions or commissural transections. As we had predicted from our earlier ablation studies, the subcortical pathway from superior colliculus through pulvinar to IT cortex turned out to be unimportant for the receptive field properties of IT neurons. By contrast, the cortical pathway from striate through prestriate to IT cortex proved to be essential, since interruption of this pathway by a unilateral striate removal or by section of the forebrain commissures eliminated the corresponding visual input to IT cells.

We have now demonstrated this functional pathway autoradiographically using the [^{14}C]deoxyglucose method developed at NIMH by Sokoloff and his group. In collaboration with Kennedy in Sokoloff's laboratory we have found by comparing a blinded and a seeing hemisphere in the same monkey that the

visual system can be clearly traced from the striate through the prestriate to the inferior temporal cortex on the basis of differential hemispheric uptake of the deoxyglucose during visual stimulation.

The results strongly support the view that the visual discrimination functions of IT cortex depend on the information it receives from the striate cortex via a relay in the prestriate. Yet there is still considerable dispute concerning this proposed relay, since even massive prestriate lesions may leave visual discrimination relatively unimpaired. To resolve this apparent discrepancy we have undertaken two new experiments. In one we are delineating by neuroanatomical techniques the entire striate-receptive area within the prestriate cortex as a preliminary to removing it in a "disconnection" experiment. In the second we are trying to determine whether even small prestriate lesions might "disconnect" IT cortex from corresponding parts of visual field by testing visual discriminations confined to those fields in monkeys that have been trained to maintain fixation. To monitor this fixation accurately, we are designing with the aid of Technical Development an infrared, corneal-reflection device.

According to the unit recording studies nearly two-thirds of IT neurons receive information from both hemispheres (i.e., from both the left and right visual half-fields). We have therefore proposed that IT cortex plays an important role in stimulus equivalence across the visual fields. This is the phenomenon in which a stimulus is recognized as the same regardless of its retinal position, even if the different retinal loci project to different hemispheres. To test our hypothesis, we prepared monkeys with bilateral IT ablations combined with section of the optic chiasm, and trained them first with one eye and then with the other eye on a series of visual pattern discriminations. Unlike control monkeys (with either IT lesions alone or chiasm section alone), who can perform immediately with the second eye any discrimination that they have learned with the first, the experimental monkey had to learn each discrimination anew with the second eye. These results provide strong evidence that stimulus equivalence across the visual half-fields depends on the convergence of their projections onto single IT neurons. We are planning to test a further prediction from this hypothesis, namely, that IT lesions will prevent transfer of a visual pattern discrimination from one part of a visual half-field to another. The method involves training monkeys whose maintained fixation is being monitored as described earlier to discriminate a pair of patterns in one quadrant of a visual half field and then test them for transfer to the other quadrant. Transfer should occur, as before, only if IT neurons are present to mediate the spatial convergence of visual information. In a related electrophysiological experiment we will test whether single neurons are indeed activated by their preferred stimuli irrespective of stimulus position in the visual field.

This spatial convergence upon single cells provides a mechanism that is essential for discrimination learning under normal viewing conditions. It insures that the same central visual cells will be activated from one exposure of the stimulus to the next despite the fluctuations in distance, direction and angle of regard that lead to stimulation of different populations

of geniculostriate cells on different presentations. But spatial convergence alone is insufficient to insure reactivation of the same central visual cells unless it is associated with a mechanism for temporal convergence. That is, a given stimulus must excite the same visual neurons on a second occasion as on the first if there is to be any summation of the effects of experience or training. We now have evidence that the IT cortex does indeed contain a mechanism for temporal convergence, as indicated by the following experiments on visual recognition.

Monkeys that are shown an object once will recognize it as familiar several minutes later, as indicated by the fact that they will avoid it in favor of another object that is completely novel. Thus, somewhere in the visual system the single presentation of a complex stimulus leaves a trace against which a subsequent stimulus presentation can be matched. If it does match, i.e., if the original neural trace is reactivated, there is immediate recognition of familiarity, and the behavioral consequence described above ensues. The area in which the neural trace is first established turns out to be the anterior IT cortex, since removals here but not elsewhere abolish the animal's ability to recognize an object that it has seen once just a few seconds before. Apparently, the anterior IT cortex contains the traces laid down by previous viewing, and these serve as control representations against which incoming stimuli are constantly being compared. In the process, old traces may either decay, be renewed, or even refined, and new traces are added to the store. An experiment is now being designed to study these newly localized visual traces at the single-unit level. The question to be addressed is whether or not a neuron that at first responds equally to two similar stimuli can be induced to respond differently to them by training the animal to attend to their physical differences.

The evidence that has been cited favors a sequential activation model for object vision. Information that reaches the striate cortex is transmitted for further processing to the prestriate cortex, and from there to the IT area. Can this model be extended beyond vision per se to encompass stimulus-reward association learning? Translated into functional anatomical terms the question is whether visual objects might gain motivational and emotional significance through an interaction between the IT cortex and the limbic system. Some preliminary findings supported this possibility. Earlier we had shown that equally severe deficits in object discrimination reversal learning are produced by bilateral removal of IT cortex and by bilateral removal of the temporal pole and amygdala (TPA), a limbic region to which the IT cortex projects. To test whether the similarity in outcome reflects a truly functional relation between the two areas, we prepared monkeys with an IT removal in one hemisphere, a TPA removal in the other, and a transection of the anterior commissure. The effect on object reversal learning was the same as that of the bilaterally symmetrical lesions suggesting that section of the anterior commissure had disconnected the intact TPA region on one side from the intact IT cortex on the other. Section of the corpus callosum instead of the anterior commissure was ineffective. The results thus supported the proposal that the process of associating visual stimuli with reinforcements depends in part on a visual-limbic pathway with a link in the IT cortex.

We are now extending those studies to exploit this new evidence of a visual-limbic interaction. First, we have undertaken a neuroanatomical investigation to define exactly the projections from IT cortex onto limbic structures; and second, we have extended and refined our behavioral assessment by replacing the discrimination reversal problem with a one-trial associative memory test analogous to the one-trial visual memory test described earlier. Whereas only IT lesions had impaired performance on the visual memory test, results with the associative memory test have indicated equivalent impairments after both IT lesions and lesions limited to the amygdala (which we find receives a direct IT projection). An attempt will next be made to disconnect the amygdala from its IT input and then test the effects of this disconnection on associative memory.

Concurrently we will be testing directly whether the amygdala is involved in the establishment of stimulus-reward associations. The study involves measuring activity of single units in the amygdala while an animal performs a visual discrimination reversal. Positive evidence for our hypothesis would be provided by a finding that the discharge frequency of a given unit depends on the reward value of the stimulus (A+ vs A-) and not on its sensory quality (A vs B). The converse results, i.e., sensitivity to the sensory and not to the affective quality of the stimulus, would be expected of units in the IT cortex, for according to our model this tissue is an intrinsic part of the visual system and concerned with purely visual processing. Preliminary results have been gathered thus far on units in the IT cortex, and, as predicted, many of the neurons here discharge selectively to visual patterns - one cell preferring one subset of patterns, another cell preferring another subset, and so on - whereas only a few of these neurons appear to code the reward value of the patterns. Whether such a code is carried by a higher proportion of neurons in the amygdala still remains to be determined.

The results of the studies described in this section so far suggest that visual discrimination habits depend on an extended, polysynaptic, cortical visual system as well as on associated limbic areas. But are these two systems acting together a sufficient substrate for such habits, or does the rest of the cortex also play a significant role? To examine this question we restricted vision to a single hemisphere and then removed all the cortex from this seeing hemisphere except for the visual and limbic systems. Unexpectedly, the operated animals appeared on awakening to be completely blind, and this total blindness lasted for one to several months, abating, if at all, only very slowly and only in part. Yet, the geniculostriate pathway in these animals seemed to be intact as judged both by visual evoked potential studies (performed in collaboration with Buchsbaum, NIMH) and from the subsequent histological examination of the brains. We are currently investigating whether this dramatic phenomenon is due to diaschisis of the visual system or to a visual-motor disconnection, the two most likely alternatives so far.

Cortical Mechanisms in Spatial Perception

The visual pathway described in the previous section is important mainly for object vision, that is, for the analysis and coding of the physical dimensions of visual stimuli and for their identification through associations

with other events, particularly motivational and emotional ones. There is a second major visual pathway out of the striate cortex, however, and this one appears to play an important part in spatial vision, that is, in the analysis of the spatial location of objects and in the use of this information for the guidance of movement. The first pathway is directed ventrally to the temporal and frontal limbic systems. By contrast, the second pathway is directed dorsally and appears to provide access to the motor system. The evidence in support of this dichotomy of cortical visual pathways and the reasons for categorizing the second one as primarily spatial rather than purely visual in function come from recent experiments in our laboratory on the parietal and frontal lobes.

We had found earlier that bilateral posterior parietal lesions produced severe impairments on a spatial test in which the animal was required to choose a response location on the basis of its proximity to a visual "landmark". The effective lesions were large, invading not only inferior parietal but also dorsolateral and medial prestriate tissue. To test whether the responsible damage was, in fact, to the prestriate rather than to the parietal cortex, new experiments have been performed with more selective lesions. The results have failed to reveal any localization within the larger region, and have indicated instead that each part contributes to the function equally. The findings thus give added support to the view that the inferior parietal lobule, rather than being a modality-specific tactual association area as was once thought, is probably a multimodal area with a significant part played by vision. To test for the dependence of this area on visual input, a crossed-lesion study has been undertaken. A posterior parietal lesion in one hemisphere is combined serially with a total striate removal in the other, and these are followed by callosal transection. Animals trained on the landmark test are severely impaired by the contralateral removals alone (performed in either order), and once recovered they are not re-impaired by the callosal lesion. The results suggest that the inferior parietal cortex, like the inferior temporal, depends heavily on striate input for its visual function; but unlike the inferior temporal, the posterior parietal does not seem to receive bilateral visual input. Thus, each posterior parietal area may be organized largely as a substrate for contralateral spatial function, and this could account for the symptoms of contralateral spatial neglect that have so often been reported after unilateral parietal injury.

The spatial functions of the parietal area have been categorized on the basis of our studies as "allocentric", where spatial location is defined in terms of referents external to the animal. Another form of spatial perception is "autocentric", where location is defined with reference to the animal's own body. Evidence is accumulating that this form of spatial orientation is dependent on dorsal prefrontal cortex, and that impairment in autocentric localization after dorsal prefrontal damage is the basis for the frontal animal's impairment on spatial delayed response tasks. (The dissociation between an allocentric and an autocentric spatial mechanism derives in part from our finding that parietal and prefrontal lesions each impair one mechanism but not the other.)

Crossed-lesion experiments like those described above have shown that the

dorsal prefrontal cortex also depends on striate input for its visual spatial function. Evidence that the function is selectively spatial comes from a recent experiment in which animals with selective prefrontal removals were compared on a series of nonspatial visual memory tests. These included delayed object alternation, delayed color matching, and delayed object matching. In every case, animals with lesions confined to the cortex in the principal sulcus quickly reattained criterion performance, in sharp contrast to their notoriously poor performance on spatial visual memory tests. In another experiment we obtained evidence that autocentric spatial localization is probably not a purely kinesthetic function. On a test that required precise judgments of the extent of head or hand movements, prefrontal lesions alone produced little or no impairment. Since it is thus possible to spare kinesthetic discrimination ability while at the same time severely impair spatial perception, it is clear that the two abilities must be at least partly independent. At the same time, other evidence was obtained that with sufficiently large frontal lesions, which included both dorsal prefrontal and dorsal premotor cortex, a severe kinesthetic impairment could be produced. The findings thus point to a substrate for kinesthesia that is close to, yet separate from, the substrate for spatial perception. Indeed, it is reasonable to suppose that the system for autocentric localization depends on input not only from vision, but from kinesthesia and many other modalities, and, further, that the dorsal prefrontal area, like the posterior parietal, is a center for convergence of multimodal information organized in terms of some spatial coordinate system. The hypothesis suggests a new direction for electrophysiological studies of these dorsal cortical association areas.

It was noted above that the outputs of these dorsal association areas may be directed toward the cortical motor system and thus permit spatial guidance of movements. Disconnection experiments involving the spatial and motor systems, like those described earlier involving the visual and limbic systems, would provide a direct test of this proposal.

Development of Brain and Behavior

A major goal of this laboratory continues to be the elucidation of the neural mechanisms responsible for the development of normal behavior and for the expression of plasticity in neuropathological development. Our approach is broadly multidisciplinary, involving behavioral, neuroanatomical, electrophysiological, endocrinological and biochemical techniques. Our experimental subject is the rhesus monkey, whose appropriateness as a model of human development is unexcelled. For studies of development, in particular, the protracted course of postnatal maturation exhibited by rhesus monkeys permits greater resolution of the timing, sequence, and correlation of structural and functional events than is possible, for example, in rodents or other laboratory animals.

Cryogenic Depression of Cortex at Different Stages of Development. Our previous work in this area has shown that specific regions of the primate cortex are functionally immature at birth and develop their functions according to widely different time-tables. However, the immature monkey differs from the adult not only with respect to cortical immaturity but with respect also

to cortical-subcortical organization. This year, with Dr. Garrett Alexander, we have employed cryogenic cooling techniques and electrophysiological recording to further describe the changes in cortical-subcortical organization that take place in ontogeny. Previous studies by Dr. Alexander have shown that the frontal cortex can be inactivated in adult monkeys by cooling through cryoplates implanted over the dura mater of the prefrontal cortex. This technique has several advantages over the ablation method including the avoidance of secondary degeneration in structures connected with the frontal cortex and also the possibility of functional effects that are reversible - allowing each animal to serve as his own control. In the adult monkey, localized cooling of the prefrontal cortex results in behavioral deficits classically associated with removal of this cortex but these deficits disappear as soon as the cortex is returned to its normal temperature. Furthermore, cooling has been shown to inhibit the firing of neurons in the dorso-medial nucleus of the thalamus, in association with decrements in behavioral performance. Studies identical to those carried out in the mature monkey are now being conducted in normal monkeys at various stages of postnatal development. Our studies show that cryogenic depression of the dorsolateral prefrontal cortex does not result in adult-typical deficits on a spatial-delayed response task in monkeys 31-34 months of age, that is, close to sexual maturity. The results obtained with the reversible lesion procedure in normal monkeys are remarkably similar to those obtained in monkeys with permanent brain damage and thus provide evidence that cortical cooling is a powerful approach to the study of regional maturation in the primate brain. Moreover, the consistent results observed with both the ablation and cooling procedure provides strong evidence that the dorsolateral cortex does not become fully functionally mature until well into the second year of life. Unit activity has been recorded in the dorsomedial nucleus of the thalamus and in the caudate nucleus - subcortical structures with which the frontal cortex is connected. Recordings from these structures in behaving monkeys at different stages of development and under conditions in which the descending influence of cortex can be controlled (through hypothermia) should reveal the extent to which subcortical mechanisms can function more autonomously in the young than the old, a principle of central nervous system organization in normal development suggested by previous research, and one which could help to explain the remarkable sparing of function observed after early cortical injuries.

Sexual Dimorphism in Cortical Structure and Function. A major effort continues to be devoted to examining the possible role that gonadal hormones may play in regulating the differentiation of cortical mechanisms. Previous work in this laboratory has provided evidence that the orbital prefrontal cortex becomes functionally mature at different times in males and females, since cognitive defects can be induced by orbital lesions at younger ages in males than in females. Currently we are studying the behavioral and biochemical consequences of postnatal injections of testosterone in developing female monkeys. Our evidence indicates that (1) androgen accelerates the development of orbital prefrontal function, suggesting that gonadal hormones may play an inductive role in the differentiation of cortical mechanisms similar to that played in the differentiation of hypothalamic mechanisms; and (2) the critical period for the influence of androgen on functions of the

central nervous system in primates extends into postnatal life. Subsequent to further behavioral studies involving analysis of their psychosocial and sexual development, these hormonally treated monkeys are sacrificed and their brains analyzed, along with brains of normal males and females, for distribution and biosynthesis of biogenic amines.

The possibility that there are neural differences between males and females at the cortical level under the control of gonadal hormones at critical periods in development is of extreme importance for understanding the normal differences in cognitive and social development of the two sexes as well as for understanding disturbances in psychosexual orientation that may occur as a consequence of exposure to an inappropriate hormonal milieu during gestation such as adrenogenital syndrome and progestin-induced pseudo-hermaphroditism. Future plans for work in this area include behavioral and biochemical studies of prenatally-androgenized females and prenatally feminized males (chemically gonadectomized with an androgen antagonist, cyproterone acetate). Prenatal studies are warranted because the peak sensitivity of cortical tissue to gonadal hormones may occur in the second trimester of pregnancy during which, in the primate, corticogenesis is completed.

Behavioral and Anatomical Consequences of Prenatally-Induced Brain Damage. The presumptive dorsolateral prefrontal cortex was resected bilaterally in a fetal rhesus monkey at E106 (106th embryonic day) with subsequent replacement in utero and cesarian delivery near term (165 days). Cognitive functions dependent upon the integrity of this neocortical region were assessed during postnatal development. The monkey was sacrificed at 2½ years of age and its brain fixed by vascular perfusion and processed for subsequent cytoarchitectonic and hodological analysis. The prenatally operated monkey grew to 2½ years of age without exhibiting any gross sensorimotor deficits or evidence of impairment on the classical spatial delayed response problems which depend critically upon the integrity of the dorsolateral prefrontal cortex in mature monkeys. Its performance on these tests was within the range of unoperated monkeys rather than severely impaired as in the case of monkeys with the same cortical areas removed as juveniles or adults. The lesion performed at E106, when brain fissuration has barely begun, were bilaterally symmetrical and similar in size and topography to those performed after birth when the brain has attained the adult fissural pattern. However, the surface of the telencephalon in the monkey operated upon as a fetus exhibited marked changes in gross morphology: anomalous sulci appeared bilaterally on the lateroventral surface of the frontal lobe and some unusual sulci were also observed in locations as remote as the occipital lobe. The cytoarchitectonic composition of the cortex lining these sulci displayed the basic six-layered pattern of primate neocortex. Perhaps the most significant finding with regard to a possible explanation of functional plasticity is that the parvocellular subdivision of the medio-dorsal thalamic nucleus, which normally projects to the dorsolateral prefrontal cortex, shows little evidence of neuron loss, indicating perhaps that the parvocellular neurons have found some other cortical target for projection. This sector of the dorsomedial nucleus always degenerates following comparable prefrontal lesions performed after birth. Thus, removal of the

dorsolateral prefrontal association cortex in a nonhuman primate a full two months before birth can be followed in postnatal life by extraordinary preservation of behavioral function as well as by significant alteration in the gross morphological characteristics and thalamo-cortical connections of the brain. We are currently engaged in studies to determine the precise neuroanatomical mechanisms underlying this striking example of neurobehavioral plasticity.

Neurochemical Correlates of Normal and Disordered Development in Primates.

As mentioned, behavioral testing following ablation of the dorsolateral and orbital prefrontal cortical areas in the developing monkey have now shown that these two cortical subdivisions develop their functions according to different timetables. Also the orbital cortex becomes functionally mature earlier in males than in females. We need to know more about the maturational status of the brain at different stages of development in order to fully understand the ontogeny of behavior and neurobehavioral plasticity in the developing primate. Measures of neurotransmitter levels and rates of synthesis give an indication of synaptic mechanisms in normal development and provide a baseline against which to evaluate events following acute or chronic brain damage.

Three monoamine putative neurotransmitters are of interest in these studies: an indoleamine, 5-hydroxytryptamine (serotonin), and two catecholamines, noradrenaline (NE) and dopamine (DA). Normal neurochemical profiles have been measured in various cortical and subcortical structures of rhesus monkeys at 2 days, 8 months, and 2 years of age or beyond. Levels and biosynthetic activity follow different developmental patterns which are specific to structure and amine. For example, the brain stem shows higher monoamine levels and rates of synthesis at birth than during adulthood. In contrast the general pattern of catecholamine development in other subcortical structures such as caudate, putamen, hypothalamus and hippocampus is a gradually increasing catecholamine level from birth to maturity with rates of synthesis increasing in proportion to concentration. The indoleamine, serotonin, on the other hand exhibits higher levels in some of these structures (caudate and putamen) at birth than at 8-months of age or adulthood. In cortex, only prefrontal areas contain an adult concentration of dopamine. The other amines increase in linear fashion from birth to maturity in prefrontal and other regions of the cerebral mantle. It is likely that the high dopamine levels in newborn prefrontal areas reflect maturational events rather than utilization for synaptic transmission.

Monoamines exhibit a differential distribution in the cerebral cortex of both infant and adult monkeys. A major finding is that monoamines are not evenly distributed in the primate cerebral cortex but instead exhibit a definite gradient of distribution specific to each amine. Dopamine concentration is highest in prefrontal cortex and declines linearly in a rostral to caudal direction. Although serotonin is rather evenly localized, its deaminated product, 5-HIAA, is distributed in almost mirror image fashion to that of dopamine. Thus, the concentration of 5-HIAA is highest in visual cortex and decreases in a caudal to rostral direction. Both DA and 5-HIAA show high concentrations in temporal cortex. In contrast to both DA and

5-HIAA, NE exhibits a more erratic pattern of distribution. A notably high concentration of NE is found in the somatosensory cortex. Anterior inferior temporal cortex and superior temporal cortex also contained substantial amounts of NE which were higher than that in the visual and posterior infero-temporal cortices, on the one hand, and that in the frontal cortex, on the other. There was thus the suggestion of peak concentrations of NE located in the central cortical zones with NE gradients diminishing along both the anterior and posterior axes. Until now there has been little recognition of the differential concentrations and turnover of monoamines in various areas of primate cortex. These findings as well as the evidence for differential rates of development (Brown and Goldman, 1977) encourage the detailed examination of the neurochemical basis of higher behavioral processes and their elaboration. In particular, the role of serotonin in visual function, norepinephrine in somatic sensation, and dopamine in cognition need to be explored.

To investigate the hypothesis that the developing brain reacts differently to damage than a fully mature brain, levels and synthesis rates of monoamines in various cortical structures have been measured eight months following bilateral orbital lesions in infant and adult monkeys. The monoamine levels and synthesis rates have been compared to data from normal animals of the same age group. Two major differences in response to damage have so far been observed between infant-lesioned and adult-lesioned animals: 1. At the site of damage, there is an increase in catecholamine synthesis in infant brains, and a decrease in synthesis in adult brains. 2. In thalamus of the infant lesioned brain, there is an increase in dopamine content and a 50% decrease in serotonin synthesis rate. A different response occurs in the thalamus of adult-lesioned brain. Here dopamine content does not increase and the serotonin synthesis rate increases 200%. Further work is in progress to identify the biochemical alterations that occur following brain damage. The findings from such studies may eventually suggest a pharmacological approach to ameliorating some of the symptoms of brain injury in the young and the old.

The Role of Biochemical Systems in Determining Cognitive Behavior

If the principal sulcus in the monkey is aspirated, he cannot perform above chance level on a cognitive task such as delayed alternation. The question may be asked whether or not any changes in a biochemical system in the brain can be identified with this behavioral effect. Accordingly, specific neurotoxins were used to deplete one or several of the endogenous neurochemicals and the behavior of a monkey monitored on the delayed alternation test.

So far our efforts have centered around 6-hydroxydopamine (6-OHDA). Pilot work indicated that 6-OHDA injected directly into the principalis area of the brain (injections spaced every 3 mm. along the superior and inferior banks, 100 µg./site) depleted NE better than 90 percent and DA better than 70 percent of normative values, one week after surgery. The depletion was restricted to the principalis region, and only NE and DA were significantly affected. These animals showed profound long-term NE depletion of better than 95 percent, DA depletion of 30 to 40 percent, and 5-HT depletion of 50 to 60 percent in

the principalis region. Outside of the principalis monoamine levels were near normative levels except for NE which was depleted 40 to 50 percent throughout the frontal cortex. Subcortical structures, in contrast, were largely unaffected by the 6-OHDA treatments.

Data on 11 animals at various stages of training and testing indicate that the 6-OHDA-treated animals, despite their massive NE depletion in prefrontal cortex, do not show the gross debilitation in delay-alternation performance seen in surgically lesioned animals. Rather they show a subtle but consistent degradation of performance. The animals have been trained and tested on a variable delay alternation task using a range of delay values between 5 and 120 sec. This procedure permits the plotting of a delay-performance function, which describes the fall in proficiency that accompanies the longer delays. The 6-OHDA treatments result in the animals performing quite well at short delays but doing somewhat worse than expected on the progressively longer delays. Thus NE in this region of prefrontal cortex appears to play only a small role in mediating functions that control delayed-alternation performance.

Most recently we have combined the 6-OHDA treatments with pretreatments of desmethylinipramine (DMI). DMI prevents reuptake of catecholamines into noradrenergic neurons. The result is that DMI protects noradrenergic neurons from the cytotoxic effects of 6-OHDA, and because of the larger pool of free 6-OHDA available, dopaminergic neurons are destroyed to a much greater extent. Although the biochemical analyses have not yet been performed, the DMI - 6-OHDA - treated animals should have near normal NE levels and greatly depleted DA. One of these animals has been tested behaviorally and while completely normal in visual pattern discrimination it has shown a profound deficit in delay-alternation performance, very much reminiscent of surgically lesioned animals. An attempt will now be made to establish that DA is an important factor in mediating the cognitive functions executed by the prefrontal cortex, while NE is of secondary importance.

If these neurochemicals are indeed important for the normal functioning of prefrontal cortex, then the question may be posed whether performance of the depleted animals can be restored to pre-operative levels given some therapeutic agent that will restore catecholamines to normal values. To date we have been successful in returning the 6-OHDA animals' delay performance to completely normal levels by treating them with d-amphetamine or L-DOPA before testing. This therapeutic effect is completely reversible.

Anatomical Correlates of Development and Plasticity

Studies of Normal Development. Concurrently with the behavioral, electrophysiological and biochemical-endocrinological studies described above, we are conducting experiments aimed at elucidating the structural basis of normal and abnormal behavioral development. Our anatomical studies using silver impregnation techniques have confirmed findings obtained with behavioral and electrophysiological methods in indicating immaturity of the pathways from the orbital and dorsolateral prefrontal cortex to subcortical structures like the caudate nucleus up to six months of age and beyond. Our findings using radioactive tracer methods include: (1) The cortico-cortical

and commissural efferents of the frontal association and motor cortex terminate in a distinct columnar pattern. Since, until now, columns have been observed exclusively in sensory cortex, findings in association and motor cortex indicate that columnar organization is a general feature of the mammalian neocortex. Further studies aimed at elucidating the source, mode of distribution and pattern of innervation of extrinsic connections in cortex are in progress. (2) Prefrontal cortico-cortical and commissural efferents terminate in all layers of target cortex, a feature of laminar distribution which distinguishes the frontal association cortex from sensory cortical areas. (3) Prefrontal cortex projects heavily upon the caudate nucleus whereas connections from motor cortex are sparse. This finding provides direct evidence that the caudate nucleus is related both anatomically and functionally more to the prefrontal cortex than to the motor cortex, and necessitates a revision of long-held conceptions about caudate function. A detailed analysis of the cells of origin of these pathways are now underway. (4) Specific regions of the prefrontal cortex project all the way to the tail and body of the caudate nucleus as well as to its head. This distribution of prefrontal striatal connections revealed by autoradiographic methods changes the picture of topographic relationships between the cerebral cortex and the caudate based on silver degeneration methods. (5) Prefrontal projections to the caudate nucleus are stereometric, indicating that segregation of afferents applies to subcortical as well as to cortical structures and is a general feature of central nervous system organization. In further studies we plan to reconstruct the stereometric input to the caudate nucleus. (6) Columnar organization in frontal cortex and stereometric innervation of the caudate nucleus are well-delineated in the newborn monkey. The same features are present but not as pronounced in older monkeys given equivalent doses of radioactive amino acids. The differences between immature and mature monkeys may relate to a number of factors including developmental differences in axonal transport mechanisms, packing density of neurons and blood-brain barrier. We are exploring these factors as we continue to study the changes in connections during postnatal development.

Studies of Central Nervous System Reorganization. A second major question that is being approached anatomically is that of the presumptive reorganization of the central nervous system subsequent to early cortical injury. Though central nervous system plasticity is presently an area of active research interest there has so far been no strong evidence to indicate that postlesion axonal growth or collateral sprouting is functionally adaptive. Our previous studies of behavioral recovery in monkeys sustaining orbital prefrontal injuries in infancy show that the monkey is an excellent animal model of behavioral plasticity in development and one highly suitable for studies of neural reorganization. We are therefore employing both the Fink-Heimer silver impregnation technique and autoradiographic methods to examine the possibility that there are anomalous connections in the brains of monkeys, now adults, that had received bilateral orbital prefrontal lesions as infants, and exhibited restitution of functional capacity to an extraordinary degree. These longitudinal studies are leading to the conclusion that behavioral recovery following early brain injury in primates cannot be attributed to the capacity of the developing nervous system for postlesion axonal growth. In this respect, primates may have evolved

adaptive mechanisms to cope with traumatic injury different from those exhibited by small mammals and invertebrates, in whom generation of anomalous connections or regeneration of damaged neurons is possible.

Annual Report of the Laboratory of Psychology and Psychopathology

National Institute of Mental Health

David Rosenthal, Ph.D., Chief

July 1, 1976 to September 30, 1977

This annual report will be the last that I shall submit as a Lab Chief. My tenure as Lab Chief has covered more than ten years. It seems to me that serving in this capacity for more than a decade is well beyond the call of duty. I have submitted my resignation as Lab Chief for two major reasons: 1) To have a change of leadership in the Laboratory, possibly with new added research directions, as well as with the past and current research that has been the hallmark of this Laboratory; 2) to be free of the responsibilities and problems and non-research activities that consume much time away from research.

This decision was not made lightly, and I regret having to give up some of the many benefits that accrue to the Lab Chief position, and I regret as well the impact on the Laboratory that occurs inevitably when a Lab Chief resigns.

Nevertheless, the Laboratory has its own internal strengths, and it will continue to contribute fruitfully to our understanding of mental illness.

Dr. Philippe Khouri will leave the Laboratory for a new position elsewhere, and we will feel his loss; but the Laboratory has also been fortunate to have Dr. Herbert Weingartner join us as a full-fledged member. It is likely, too, that two long-standing members of the Laboratory will leave us at about the end of the calendar year. Thus, the Laboratory will need at least two positions to fill the gap, as well as one or more positions that the next Lab Chief will require. As of this writing, efforts are ongoing to recruit a new Chief.

The Laboratory faces new problems as well, in that it will have to move out of the Clinical Center and reside in Building 31. Some members of the Lab may have lab space in the Clinical Center, but most will not. In any case, the next Lab Chief will have to supervise a somewhat fragmented Laboratory, at least for a while. However, when the Ambulatory Care Facility is completed, this Laboratory should benefit appreciably, since many of our subjects are ambulatory.

During the past year we have carried out the following research:

Animal models for study of psychopathology

A major goal of our research is to develop animal models for the study of psychopathology. We continue to maintain several sublines of the BALB/c inbred mouse strain which are being used to investigate the intra-strain difference between the NIH and JAX sublines in aggressive behavior reported by Ciaranello and Lipsky. We have been unable to date to replicate this study of catecholamine-synthesizing enzymes and aggression, but we are continuing attempts to unravel the environmental variables which may cause this inconsistency.

We continue to maintain a large stock which we developed as a result of the H₂ mouse strain developed by McClearn from the cross breeding of inbred mouse strains. This genetically segregating population is used to study of MAO activity and general motor activity levels. The results of these studies suggest that the percentage of MAO activity in both liver and brain as exhibited by inhibition curves does not differ from the pattern reported in rat tissues. The possibility of assaying mouse platelet MAO activity continues to be investigated. When preliminary investigations of variability in mouse MAO activity have been completed, we hope to be able to measure the genetic correlation between brain and platelet MAO activity.

Normal social interactions in small groups of mice have been and are being observed. We are attempting to develop new behavioral tests in a social situation for the assessment of drug response. We are particularly interested in developing an appropriate test for the effects of LSD, amphetamines, phenothiazines and MAO inhibitors on mouse behavior.

Schizophrenia

A study of the offspring of schizophrenics revealed:

- 1) An increased frequency of two types of behavioral abnormalities, hyperactivity and "socio-emotional immaturity" (fearfulness, dependency, low frustration tolerance, shyness) among the male offspring of schizophrenics compared to controls.
- 2) The hyperactivity in these children occurs most often when the mother is schizophrenic and the father has psychopathic traits (alcoholism and/or abusiveness).

An effort was made to devise a strategy for the study of the role of biological factors in psychiatric illness. This strategy assumes that schizophrenia and other psychiatric illnesses may be heterogeneous and thus permits the evaluation of a biological abnormality as only a percentage of patients with a certain diagnosis. Basically the strategy is as follows:

- 1) Select patients with psychiatric illness "A" and biological abnormality "X."
- 2) Examine all relatives for "X."
- 3) If "A" is found only among the relatives with "X" then this abnormality appears to be a necessary (but not always sufficient) factor for the illness in those families.
- 4) If "A" is found only among the relatives with "X" but "X" relatives are also found among the relatives of "A" relatives, then "X" abnormality may be a contributory factor.

This strategy has not been applied to schizophrenia, but we are initiating a study of low platelet MAO, abnormal personality, and life handedness in schizophrenics using this strategy.

The term biotypes is used to describe a group of individuals with a certain illness in which there is a common biological abnormality which is etiologically linked to the illness. This term is used to describe a group of individuals with a certain illness in which there is a common biological abnormality which is etiologically linked to the illness.

The term is used frequently in clinical practice, but not often in research, because it has not been adequately validated. Spitzer and Endicott of the NYS Psychiatric Institute, using the case records from the Danish adoption studies by Rosenthal, Kety and Wender, have selected out certain criteria for the diagnosis of borderline. We have worked with Endicott on the further development of these criteria, devising rating scales and an interview schedule for employing them in research.

In another project we dealt with the development of criteria that can reliably differentiate borderline schizophrenics from chronic schizophrenics and psychopathologies outside the schizophrenia spectrum. Eight items were derived. Two manuscripts were written on this project; the first dealt with the data part and the second with the theoretical aspect and its implication for future genetic and biochemical research.

In an MMPI assessment of psychopathology in the adopted-away offspring of schizophrenics, the results of the study support a genetic hypothesis of schizophrenia. Among the adopted offspring of schizophrenics, a combination of MMPI criteria and clinical diagnoses identified 22% as having a schizophrenia spectrum disorder. Only 6% of a matched control group were similarly identified ($p < .01$).

In a study of relationships among personality, biochemical, and psychophysiological variables in college students, an effort was made to determine whether individuals with extreme MMPI scores are similar to psychiatric groups with respect to low MAO, augmenting AER, and the presence of clinical symptoms. These data are not yet completed for analysis.

In studies of thought disturbance, we have developed a technique to assess an individual's use of syntax in different conditions. Our testing indicates that acute schizophrenics are able to use syntax as normals do even when the syntactic properties of a sentence are stressed with semantically confusing words. A small subsample of drug-free schizophrenics appear to have impaired syntactic ability. We are continuing to investigate these differences.

Another study involves clinical and empirical detection of simulated schizophrenia. The purpose of this project is to determine to what extent a skilled interviewer and psychometric tests can detect trained actors faking schizophrenic symptoms. This study originated in response to Rosenhan's article in Science, "On being sane in insane places." Data collection will begin shortly.

A reexamination of the conditionability data in the Denmark Adoptive Study was carried out to determine whether those subjects diagnosed as schizophrenic, or as having a schizophrenic diathesis, show differences in autonomic nervous system activity as compared to normal controls. It was found that the diagnosis of subject variable showed a number of significant interactions suggestive of a tendency toward greater electrodermal responsivity among spectrum-diagnosed subjects as compared to their matched controls. There was also a significant finding for conditionability which suggested that spectrum-subjects manifested a greater degree of conditioning than did controls. The ego strength variable differentiated subjects in terms of their response amplitude to orienting tones. Several other significant findings indicated a tendency toward response

habituation for high rather than low ego strength subjects. Both sets of results were interpreted as showing a modest but consistent trend with that of previous studies. The relative lack of significant findings for the interaction of the diagnostic and ego strength variables was also discussed.

A comprehensive review of the literature on personality factors and electrodermal arousal was also completed during this review period. This paper was specifically oriented toward evaluating the degree of consistency between theory and research on electrodermal arousal for extraversion, field dependence-independence, ego strength and sensation-seeking variables. The major findings were that type of stimulus situation tends to be important in obtaining arousal differences for personality groups, certain personality dimensions show global trends for electrodermal conditionability.

It was also found that these four Western personality dimensions show some correspondence with the Russian dimensions of strength of the nervous system and dynamism of excitation. Despite a paucity of research directly testing these relationships, there is a consistent trend in several recent reports that extraversion is associated with the strength dimension. Based upon these findings, several suggestions were made for carrying out future studies on arousability and personality.

Data analysis and report writing is continuing on a study of autonomic nervous system and attentional functioning in drug-free acute schizophrenic patients and normal controls. The major findings are greater ANS activity (as measured by skin conductance and heart rate) in the patients under resting conditions, smaller phasic and tonic increments in arousal to a variety of stimuli and tasks (even when initial levels are taken into account), reduced anticipatory activity, and slow habituation. The pattern is similar to previous results with drug-free chronic patients. These characteristics, however, were found to be pronounced only in patients who remained acutely psychotic during a 4-5 month hospitalization. Patients who recovered from the psychotic episode more quickly, despite equally severe psychopathology ratings at the time of testing, did not have as deviant ANS or attentional functioning. In addition, this functioning did not change appreciably with clinical improvement, suggesting either that the psychophysiological changes preceded the change in psychopathology or that they are more permanent aspects of functioning, similar to traits.

An attempt to replicate and extend some of these findings is in progress. In addition, since the patients in the current study are on a double-blind drug protocol, it will be possible to determine if the psychophysiological data has any predictive value for specific types of pharmacotherapy. We have also begun testing patients on the protocol to determine the effects of drugs on ANS functioning.

In other research we are attempting to discover psychological and biological relationships to ANS and attentional performance in normal subjects, partly to elucidate the findings obtained in patients. A collaborative study with LSES, (APB, and LCS in the data analysis stage concerns the interrelationships of ANS and monoamine oxidase activity, perceptual task performance, personality, mood and everyday behavior. A partial replication of this study focuses on ANS responses to stimuli of various intensities in relation to traits such as extraversion, anxiety and stimulus seeking.

We are participating in a study with APB on the effects of d-amphetamine on the ANS and attention in normal and hyperactive children, which we hope will help us understand the "paradoxical" quieting effects of stimulant drugs. Finally, in a study about to begin with investigators from LCS, we will be testing the effects of psychological stress on concurrent determinations of ANS variables and plasma catecholamines in normal subjects and in patients with ANS related disorders, such as hypertensives and certain neurotic groups.

Other studies

A continuing purpose of our research is to specify the functional speech units in perception and production. The unit under study is the phonemic clause, a linguistically defined string averaging 7 to 8 syllables, which has been shown in a series of previous studies to have psychological as well as linguistic significance.

Our current strategy is to examine experimentally the deployment of listeners' attention. We hope to show that speech perception is a phasic process involving alternation between passive short-term memory registry during the utterance of a phonemic clause and a burst of cognitive activity at the termination of the clause.

This phasic conceptualization implies a corresponding phasic deployment of attention, outward during the registry of the clause and inward during the cognitive processing and recoding at the terminal boundary.

In a series of preliminary studies we attempted to study this hypothetical process using the cortical average evoked response as the dependent variable. In the past year we have tabled this approach and shifted to reaction time and GSR as measures of intercurrent shifts in attention. An audio tape of a dialogue is played to the subject, and he is instructed to pay attention to the dialogue and to press a key whenever he hears a click in his earphone. The clicks have been precisely placed on the second channel of the audio tape such that half of them occur at clause boundaries and half elsewhere within clauses. The experimental prediction is that the subject's mean reaction time will be longer and his GSR slower and smaller to the clicks occurring at boundaries, because his attention will be occupied with the necessary cognitive processing.

It is important to note that, if successful, this project will open an unexplored domain. No investigation of brain response to natural speech has ever been carried out. Beyond the initial normative studies lie the possibilities of investigating attentional abnormalities or deficits in schizophrenia, with particular regard to lateral specification of cortical function. It is widely believed that much of the cognitive and perceptual deficit in schizophrenia may reflect an underlying disorder of attention, specifically an impairment in the capacity to organize complex stimulus patterns and focus on the salient elements. Since the left hemisphere seems to be specialized for speech, a psycholinguistic approach to the problem of laterality and attention has considerable strategic potential, at this stage of our knowledge.

Our research on schizophrenic thought disorder has moved from issues of informational organization, semantic organization, context, to an analysis of

syntactical structures and how these might determine the characteristics of schizophrenic thinking.

The research efforts have invariably been executed within a framework which promotes a translation of clinical findings, basic biological findings, and psychological cognitive observation so that research in these ordinarily disparate areas can be reflected and used to more effectively understand both basic processes and clinical phenomena.

Other research efforts summarized here have had three broad and necessarily interrelated aims. These have included studies of 1) psychological processes that might account for the storage (learning, acquisition) of experience and the conditions that permit its retrieval at some later time, 2) the biology of information storage and retrieval, and 3) the pathological expression and changes in information processing or those that occur in response to drug-altered changes in the brain.

The research that has been pursued most recently in this laboratory under cognitive storage and retrieval mechanisms has included:

a) Studies characterizing the relationship between stimulus characteristics (e.g., imagery, encoding factors, post-stimulus arousal) and retrieval conditions that relate to specific and nonspecific encodings of stimuli.

b) Characteristics of semantic memory and its reflection in determining the encoding of events.

c) The consistency of retrieval from long-term memory as a function of stimulus encoding.

d) Cognitive processes that define information acquisition as reflected in the prediction of later recall probabilities.

e) Speed of processing, retrieval and stimulus encoding as determinants of various components of learning and memory. This has been studied by using levels of processing analyses of information storage and retrieval.

f) The nature of reinforcement in memory consolidation.

A number of projects have been generated which have explored some of the biological factors that might help account for the psychological mechanisms, as above, where these studies have been pursued in parallel with the research efforts outlined above.

a) Characteristics of memory consolidation as determined by arousal manipulations at the time of information storage, or just after acquisition. These studies have involved manipulations using behavioral techniques and with the use of drugs.

b) Drug manipulation of REM sleep and its effects on various components of mood, arousal, storage and retrieval of information.

c) State-dependent learning of information under various drug conditions including marijuana, physostigmine, amphetamine, etc. This research has been used as a particularly useful tool in defining the biological state that determines the context for uniquely storing (encoding) and retrieving events.

d) Changes in learning, memory and retrieval of language and pattern information in response to amphetamine treatment is presently being examined in a group of normal children. The focus of the study has been to examine the amphetamine cognitive response in terms of various components of storage and retrieval

processes. Clinical studies have remained the most important arena for investigating factors that might account for the acquisition and retrieval of experience. It has also become increasingly clear that the cognitive models we have developed have been particularly useful in providing us with a far fuller, more detailed picture of a variety of important clinical phenomena.

We have completed and expanded research in the following areas:

- a) We have extensively explored and researched the dementias including: Huntington's disease, Korsakoff's psychosis, Alzheimer's disease, and other "organic" disorders. At this point we feel we have built a major program for the interrelated study of the psychology and biology (e.g., neurochemistry, psychopharmacology) of the dementias. It would appear that we have been particularly successful in building and then using the bridges that might tie cognitive psychology to a biological framework and where these two broadly defined systems might jointly be productively used to study a set of discrete brain syndromes.
- b) The mood disturbances in man: We have continued to research how changes in mood might 1) be defined (scaled), 2) alter how experiencing is encoded, 3) affect the amount of information which can be learned and remembered, 4) be accounted for, in part, by mood state specific lateralization of brain cognitive functioning, 5) and be altered by drugs that alter mood state.

The purpose of one of our projects is to develop valid methods for assessing variations in perceptual capacities. Most current methods are susceptible to artifacts arising from the way individuals are tested and from their attitudes toward the variables being assessed and toward the person performing the assessment. Effects associated with psychopathology, organic disability, or drug conditions can be duplicated in normal individuals under normal circumstances. The duplication is not a matter of simulation but rather depends on attitudes and factors of which neither the subject nor the investigator is usually aware.

A strong attitudinal influence lies in the distinction subjects make between "real" and "nonreal" perceptual effects. This distinction is demonstrable in children beginning at about the age of 4-5 years and in adults of all ages. It is manifested in many behavioral functions but is particularly accessible to investigation in judgments of the visual sizes, shapes, and distances of objects, illusions, and other judgmental tasks involving the integration of stimulus information from several sources into a unitary concept or percept.

An approach, known as functional measurement theory, is being developed for dealing with such perceptual-integrative problems in a scientifically adequate way. This approach tests the validity of measurement by evaluating logically and mathematically required consistencies among judgments over a wide range of combinations of stimuli. An experiment has been designed according to the functional measurement paradigm to study the integration of size and distance information comparing instructions to judge apparent visual magnitudes with instructions to judge objective, physical magnitudes. Testing of subjects in this experiment is presently under way.

Recent research findings have been interpreted to mean that very young infants are able to integrate visual information into veridical percepts of three-dimensional external reality. On this question a collaborative experiment

with another Laboratory has been completed. Infants two to three months of age were tested for their ability to discriminate combinations of objective shape, projective shape, and slant of a visual surface. The results indicate that the infants responded to variations in proximal cues, such as differential motion parallax or retinal disparity, but did not achieve a unitary percept of a given shape at a given slant in depth. The perceptual integration of visual information into a veridical representation of external reality thus appears not to be inborn but requires experience and maturation for development.

This Laboratory has collaborated with members of the Cancer Institute on a project which includes other NIMH scientists as well. Our participation is centered around using the MMPI to predict relapse after surgical removal of melanoma tissue. Preliminary analysis suggests that psychological factors assessed with the MMPI can correctly predict relapse or non-relapse better than chance. The predictive accuracy is on a par with biological predictors. A second sample is being assessed to cross-validate our predictive functions.

These projects illustrate the usefulness of combining psychological assessment and individual differences methodology with psychiatric conceptualization of clinical research problems.

During the report period, a research project on personality and psychophysiology received Medical Board approval. The project is designed to shed information on how such factors as stimulus intensity, unchanged stimulus input, heteromodal stimulation, and sensory threshold task performance differentially affect autonomic nervous system functioning in selected personality groups. Examined also is the relative correspondence of bilateral electrodermal activity and its relation to perceptual task performance. Black and white male college volunteers are sampled in an effort to further comprehend racial differences in autonomic functioning to imperative and uncontrolled stimulus input.

To date, fifty-four subjects have been tested. Data analysis which comprises roughly three to four hours per record is nearing completion for the first session.

The proposed course of the project is to continue testing until a sufficient sample size (e.g., 150) has been attained. We also anticipate expanding the project to include female subjects and psychiatric interview information on a selected number of individuals previously tested under the original protocol.

ANNUAL REPORT OF THE LABORATORY OF SOCIO-ENVIRONMENTAL STUDIES
NATIONAL INSTITUTE OF MENTAL HEALTH
July 1, 1976 through September 30, 1977
Melvin L. Kohn, Ph.D., Chief

The time-scale of social science research is very different from that of most research done at N.I.H.--our "projects" often take a year or two to formulate, another two years or more for data collection and the preparation of the data for statistical analysis, and many years for analysis of the data and interpretation of the results. This process is further extended when the results of a cross-sectional study warrant the collection of additional data from the same respondents to make possible longitudinal analysis. Such analysis is of inestimable value in pinning down causal processes. One of our projects, on occupational experience and psychological functioning, has just completed the transition from cross-sectional to longitudinal research, with the first solid results of the longitudinal analyses newly produced. A second, on the social-structural sources of stress, is beginning that same transition, still analyzing the cross-sectional data, while simultaneously progressing well into the data-collection phase of a "follow-up" survey of the same respondents. A third project, on health orientations of children, relies on multiple sources of data rather than on longitudinal analysis for making causal inferences; this project is far into the analysis and reporting of its results.

OCCUPATIONAL EXPERIENCE AND PSYCHOLOGICAL FUNCTIONING

This study has a long and productive history, summarized in the annual reports of the past several years. The principal investigators are Melvin Kohn and Carmi Schooler, together with Joanne Miller and Karen Miller, who are here on extended post-doctoral fellowships.

The main goal of this research is to assess the effects of occupational conditions on psychological functioning. The research began with structured interviews of 3100 men, a representative sample of all men employed in civilian occupations throughout the United States. These interviews were conducted to our specifications by the National Opinion Research Center of the University of Chicago in 1964. In 1974, the National Opinion Research Center conducted follow-up interviews, again to our specifications, with a randomly-selected one-fourth of the men who had participated in the original survey. A nearly identical interview was conducted with the wives of presently-married men and, wherever a man had one or more children in the age-range 13 through 25, a similar interview was conducted with a randomly-selected child.

From these longitudinal data, we hope to assess with some precision the magnitudes of the reciprocal effects of occupational conditions and various facets of psychological functioning. The study of the wives is designed to ascertain whether occupational conditions affect men and women similarly. These data should also enable us to assess the effects of men's occupational experiences on their wives' psychological functioning and of women's occupational experiences on their husbands' psychological functioning, in each case taking account of the individual's own occupational experiences. The study of the children is designed for exploratory analyses of the effects of parental experiences, values, and practices on their children's psychological development, as well as of the children's own educational and occupational experiences on their own psychological development.

During this past year, our major research efforts have been focused on four objectives: (1) Analyzing the reciprocal relationship between the substantive complexity of men's work and their intellectual flexibility; (2) Developing measurement models for other dimensions of occupational structure and other facets of psychological functioning; (3) Assessing the effects of occupational conditions on the psychological functioning of women; and (4) Analyzing the determinants of social mobility.

1. The reciprocal relationship between the substantive complexity of men's work and their intellectual flexibility. Our longitudinal assessment of the reciprocal relationships between occupational conditions and psychological functioning has thus far focused on one pivotal dimension of occupational structure, the substantive complexity of work, and one pivotal dimension of psychological functioning, intellectual flexibility. We chose the substantive complexity of work as the occupational condition to be assessed in this analysis for three reasons: Substantive complexity is a central element of occupational structure--an important determinant of occupational self-direction and an important "structural imperative" of the job. Moreover, substantive complexity is as strongly correlated with psychological functioning as is any other dimension of occupation we have examined. Finally, we have excellent descriptive information about the substantive complexity of work, which we have been able to validate by comparison to the objective job assessments of trained occupational analysts.

We chose intellectual flexibility as the aspect of psychological functioning to be assessed in this analysis in part because it offers us the greatest challenge--intellectual flexibility obviously affects recruitment into substantively complex jobs, and there is every reason to expect it to be one of the most resistant to change of all facets of psychological functioning we have measured. Moreover, intellectual

flexibility is so important a part of psychological functioning that we must not unthinkingly assume it to be entirely the product of genetics and early life experience. Rather, we should empirically test the possibility that intellectual flexibility may be responsive to the experiences of adult life.

Analyses of longitudinal data are immensely difficult, because they require the development of "measurement models" that separate unreliability of measurement from real change in the phenomena studied. For example, we need to be able to assess how much of the apparent change in the substantive complexity of men's work represents real change in men's job conditions over the 10-year interval between the initial survey and the follow-up survey, and how much is a function of unreliability in the measuring instruments. Similarly, we need to be able to assess how much of the apparent change in intellectual flexibility represents real change in men's cognitive functioning and how much is a result of measurement error.

Recently, Joreskog and his associates have introduced powerful new procedures for using maximum-likelihood confirmatory factor analysis to separate unreliability from change in causal analysis. The essence of these measurement models is the use of multiple indicators of each of the principal concepts. Using confirmatory factor analysis, it is possible to infer from the covariation of the indicators the degree to which each reflects the underlying concept that they all are hypothesized to reflect and the degree to which each reflects anything else, which for measurement purposes is considered to be error. The measurement models must allow for unreliability in all the items that measure a concept, and also allow for these errors being correlated in repeated measurements of the same phenomena. The test of our success in differentiating "true scores" on the underlying concept from error in the indicators is how well the measurement model reproduces the original variance-covariance matrix.

We have constructed such measurement models for both the substantive complexity of work and intellectual flexibility. The models show the over-time stability of both variables to be high, that of intellectual flexibility especially so: the correlation between men's intellectual flexibility at the time of the original study and their intellectual flexibility ten years later, shorn of measurement error, is 0.93. Nevertheless, the reciprocal effects of substantive complexity and intellectual flexibility are considerable.

The effect of substantive complexity on intellectual flexibility is approximately one-fourth as great as the effect of men's 10-year earlier levels of intellectual flexibility. This effect is essentially contemporaneous: the lagged path from 1964 substantive complexity to

1974 intellectual flexibility is a statistically nonsignificant 0.05, while the contemporaneous path from 1974 substantive complexity is a more substantial and statistically significant 0.18.

An effect of this magnitude might not in ordinary circumstances be considered especially striking; but a continuing effect of this magnitude on so stable a phenomenon as intellectual flexibility is impressive, for the cumulative impact is much greater than the immediate effect at any one time. Continuing effects, even small-to-moderate continuing effects, on highly stable phenomena become magnified in importance. The effect of the substantive complexity of work on intellectual flexibility is especially noteworthy when we take into account that we are dealing with men no younger than 26 years of age, who are at least ten years into their occupational careers.

The reciprocal effect of intellectual flexibility on substantive complexity is even more impressive--surpassing that of the substantive complexity of the 1964 job. This effect is entirely lagged, i.e., it is the men's intellectual flexibility in 1964, not their contemporaneous intellectual flexibility, that significantly affects the substantive complexity of men's 1974 jobs. The longitudinal analysis thus demonstrates something that no cross-sectional analysis could show -- that, over time, the relationship between substantive complexity and intellectual flexibility is truly reciprocal. Substantive complexity has a more immediate effect on intellectual flexibility: today's job demands affect today's thinking processes. Intellectual flexibility, by contrast, has a delayed effect on substantive complexity: today's intellectual flexibility has scant effect on today's job demands, but it will have a sizeable effect on the further course of one's career. Cross-sectional analyses portray only part of this process, making it seem as if the relationship between the substantive complexity of work and psychological functioning were mainly unidirectional, with work affecting psychological functioning but not the reverse. The longitudinal analysis portrays a more intricate and more interesting, truly reciprocal, process.

The data thus demonstrate, beyond reasonable doubt, what heretofore could be stated as only a plausible thesis buttressed by presumptive evidence--that the substantive complexity of men's work both considerably affects, and is considerably affected by, their intellectual flexibility.

These findings come down solidly in support of those who see occupational conditions as affecting personality and in opposition to those who see the relationship between occupational conditions and personality as resulting solely from selective recruitment and job-molding. We do not deny that personality has great importance in

determining who go into what types of jobs and how they perform those jobs; in fact, our analyses underline the importance of these processes. But that has never been seriously at issue. What has been disputed is whether the reverse phenomenon--of job conditions molding personality--also occurs. The evidence of the study unequivocally supports the position that it does occur. Thus the findings bear directly on an issue central to the field of social structure and personality-- whether social-structural conditions affect personality only through childhood socialization or continue to affect personality throughout the life span. Here is clear evidence that one important facet of social structure-- the substantive complexity of work--directly affects adult personality.

In particular, this study adds to and helps specify the growing evidence that the structure of the environment has an important effect on cognitive development and that cognitive processes do not become impervious to environmental influence after adolescence or early adulthood, but continue to show "plasticity" throughout the life span. Our findings reinforce this conclusion by showing that intellectual flexibility continues to be responsive to experience well into mid-career and probably beyond. In fact, it appears that the remarkable stability of intellectual flexibility reflects, at least in part, stability in people's life circumstances. Intellectual flexibility is ever-responsive to changes in the substantive complexity of people's work; for most people, though, the substantive complexity of work does not fluctuate markedly.

This study demonstrates as well the importance of the impact of intellectual flexibility on substantive complexity. We think it noteworthy that this effect appears to be lagged, rather than contemporaneous. The implication is that the structure of most jobs does not permit any considerable variation in the substantive complexity of the work: job conditions are not readily modified to suit the needs or capacities of the individual worker. Over a long enough time, though--certainly over a period as long as ten years--many men either modify their jobs or move on to other jobs more consonant with their intellectual functioning. Thus, the long-term effects of intellectual flexibility on substantive complexity are considerable, even though the contemporaneous effects appear to be negligible.

2. Measurement models for other dimensions of occupational structure and other facets of psychological functioning. The next step in this analysis is to extend the causal model from one occupational condition, the substantive complexity of work, and one facet of psychological functioning, intellectual flexibility, to a much more general model of the interrelationship of occupational structure and psychological functioning. To this end, we have constructed measurement

models for such dimensions of occupational structure as closeness of supervision and routinization of work, and for such facets of psychological functioning as self-esteem, anxiety, authoritarian conservatism, receptiveness or resistance to change, and standards of morality. We are now in process of combining these various measurement models, so that we can estimate the correlations among the various concepts, shorn of measurement error. This step in the research process is both tedious and demanding; but it is essential. Once past this, we shall be in position to undertake the intended large-scale causal analysis of the reciprocal effects of occupational structure and psychological functioning.

3. The effects of occupational conditions on the psychological functioning of women. Formal employment is clearly becoming a more common life experience for women. The rate of female labor force participation has been steadily increasing; in 1970, over 40% of the adult female population was employed.

The 1974 follow-up survey of men in the civilian labor force included an interview with the wife of those men who were then married. Measures of psychological functioning and job histories were obtained in a form closely parallel to that of the men's interviews. We are currently using these data to assess the effects of occupational conditions on women's psychological functioning. The first phase of the analysis of the women's data has been to determine whether the questions used to measure intellectual flexibility, self-conception, values, and social orientation had the same meaning for women as for men. For example, the questions that were used to index father's values for their children have been shown to distinguish fathers who stress self-direction from those who place more importance on conformity to external authority. If we are to compare the effect of occupational conditions on men's and women's parental values, the same questions must index the same dimensions of valuation for mothers. It is possible, for example, that mothers' values might center on interpersonal relationships or aspirations instead of on self-direction and conformity to external authority. If the same questions tap an entirely different concept for women, statistical comparisons become impossible.

Our analysis shows that all the major measures of values, self-conception, social orientation, and intellectual functioning have the same factor structure for women as for men. This finding not only simplifies the research strategy of the study, but further verifies the universality of the concepts.

The next step in the analysis is to assess the impact of job conditions of formally employed women on their psychological functioning. The strategy for this inquiry, now in progress, is to

establish the overall relationship between conditions of work and psychological functioning for women, and then to investigate special circumstances that may alter this relationship. Two employment characteristics particularly relevant in analyses dealing with working women are full- versus part-time employment and occupational commitment. One might expect the effect of occupational conditions to be muted if the work is not full-time or is not of central importance to the individual.

4. The determinants of social mobility. The data of this study are also being used to study social mobility. Although a great deal of sophisticated research has previously been carried out in this area, the size of our sample and the depth and extensiveness of our data permit us to replicate and substantially extend the work of others. We can compare the mobility processes of old and young, black and white, and urban and rural populations. We are also able to enlarge considerably the list of social characteristics that may serve as predictors of attained occupational status. We do this by including in our models of the inter-generational mobility process such variables as maternal and paternal grandfathers' occupational status and such aspects of the individual's childhood as the religion and region of the country in which he was reared.

We are also in a position to take an unusually intensive look at intra-generational mobility, because we have both extensive background information and unusually detailed job histories. We can thus analyze not only the determinants of the job the individual held at the time of the follow-up study in 1974, but also determinants of the job he held at the time of the initial interview in 1964, and of the first job he held for 6 months or longer. Furthermore, since we have extensive data on many aspects of the individual's value system and psychological functioning in 1964, we can see how such characteristics affected his occupational mobility to 1974.

These analyses have not yet been carried far enough to come to firm conclusions. Analyses have also been performed on the psychological effects of mobility. Here, our results, though incomplete, seem fairly certain. We have not been able to locate any psychological effects of mobility that are independent of the effects of the individual's social origins and his present social status. Mobility, per se, seems to have little or no psychological impact.

THE SOCIAL-STRUCTURAL SOURCES OF STRESS

Leonard Pearlin's inquiry into the social-structural sources of stress is based on data collected at two times spanning a period of about four years. In 1972, interviews were conducted with a representative cluster sample of 2300 adults in the urbanized area of Chicago. These interviews sought information about three features of

people's lives: the chronic, often low-keyed, everyday problems that people encounter in their occupational, marital and parental roles; the coping strategies people employ in dealing with life-problems; and symptoms of emotional stress, anxiety, and depression. Then in 1976-77, a second wave of interviews was conducted with a subsample of 1200 people drawn from those originally interviewed. Many of the questions asked in the initial interview about chronic role problems, coping, and psychological distress were repeated, thus providing longitudinal information about stability and change in these dimensions of people's lives. The second interview also sought information about potentially distressful types of life-strains that were not included in the 1972 interviews. One type is represented in the many events and role transitions that are normative aspects of life-cycle--e.g., being married, having a child, retiring from work, etc. A second type of strain is also a concomitant of commonly experienced events, but these events are less expected and more eruptive, such as divorce, illness, involuntary unemployment, and so on.

Together the two interviews provide a large body of information about problems woven into the fabric of major social roles, the gains and losses involved in life-cycle transitions, the crises that can intrude into people's lives, coping repertoires, and psychological outcomes. The long-range goal of the research is to reconstruct from this information those elements of social experience that are most deleterious to mental health and to learn how people attempt to avoid being psychologically harmed by such experience.

The work of the past year proceeded along two lines: the continuation of the analysis of the 1972 data and the collection and preliminary examination of the 1976-77 data. Analysis of the earlier material largely centered on identifying factors that raise or lower self-esteem. The understanding of self-esteem is important within a larger study of social stress, for earlier investigation shows positive self-regard to be a barrier to the stressful consequences of life-strains.

Central to the analysis is the influence of social stratification on self-esteem. We have learned that the level of adults' self-esteem is associated with the level of their education, their occupational status, and their income. This finding is at variance with previous research, based mainly on school-age children; such research has consistently failed to find any connection between stratification and self-esteem. The present study has sought to identify why adult self-esteem, but not that of children, is influenced by social and economic inequality. One reason is that children's location in the stratificational system is not based on their own personal success or failure. In contrast, adults who are competing in society's opportunity

structures are likely to see their location as a direct reflection of their abilities and, depending on where they are, will either suffer decrements or enjoy increments to self-esteem. This is especially the case for adults who value achievement and status.

Another factor differentiating the relevance of stratification to the self-esteem of children and adults is the nature of adults' occupational experience. Being closely supervised by others and having little opportunity for occupational self-direction are known from other research conducted by Pearlin and by other members of this Laboratory to be inimical to self-esteem. These conditions substantially account for observed differences in self-esteem among people of different occupational status. The comparable experiences of children--in school--are associated more with age and grade in school than with their parents' social class position.

Finally, children's social interactions, centered as they are around community and school, are more likely to be with others of similar social class position than are the social interactions of adults. Consequently, children are not likely to make the same kinds of invidious comparisons, based on class status, as do adults, who are more likely to be in direct or indirect contact with people of different class position. For adults, therefore, status differences and the awareness of these differences are more likely to be related to self-esteem. In these ways, social stratification comes to affect the development of a crucially important personality characteristic of adults, but not of children.

The first analysis of the 1976-77 follow-up data, presently in its preliminary stages, is concerned with the relative impact on psychological well-being of the three types of life-strain for which we now have information: chronic role problems, role transitions, and eruptive crises. Findings are emerging that should result in some modification of prevailing thinking. One such finding is that the normative transitions that occur in occupational and family roles are the least provocative of emotional distress. This is contrary to a widely held view that the major stresses of adult development and of aging result from the gain and loss of roles. Instead, the data indicate that the durable problems of daily life and the eruptive crises with which people have to contend are more likely to create distress. A second finding contradicts another commonly held assumption-- that any life-change, regardless of its nature, results in stress because change always demands some degree of readjustment. This does not appear to be the case, for many changes--such as leaving the labor force to have children, moving to another job, or promotion--are unrelated to emotional distress. Indeed, remarriage by previously divorced or

widowed people is a change that more than any other is associated with psychological well-being.

In the coming year this inquiry will continue to analyze in detail both the psychological consequences of different life-strains in major social roles and the conditions that mediate these consequences. The study will also deal with changes captured by the longitudinal data, attempting to establish relationships between external social circumstances and inner emotional life both through space and time.

HEALTH ORIENTATIONS OF CHILDREN

The central issue in John Campbell's research is to learn how children acquire and modify their perspectives on illness and health. Information was obtained from 264 hospitalized children and their mothers by use of focused interviews, questionnaires, and rating scales. Interviews with children and mothers, and questionnaires completed by mothers provided data on their definitions of illness, reports of their own and other family members' feelings and actions when ill, their attitudes and beliefs about illness prevention and treatment, their values in selected areas not directly relevant to health, and their views about appropriate standards of behavior (i.e., what should or should not be done in circumstances pertaining to illness and health).

A major goal of this research is to clarify the manner in which health orientations are acquired. It is a premise of this study that the largely stable variations in adults' views of health and illness are to an appreciable extent the products of a social process that begins in childhood. Influences contributing to these perspectives extend well beyond those of specific health-relevant experiences; the factors at work are integral to children's socialization more generally viewed. The process leading to individual variations in health orientations is in considerable part determined by social characteristics of the child and family members, systematic differences in conditions of life, and associated differences in general parental values, role expectations, and situation-specific norms.

Analyses completed this past year have helped to clarify the process leading to individual differences in the extent to which children expose themselves to risks that might place their health at hazard, a matter of obvious importance to personal well-being. Two different types of risk taking reported by children have been considered: taking chances in active play that might lead to physical injury, and risking illness by failing to follow simple hygienic or health maintenance routines. These are logically and empirically related types of risks, and they share some antecedents. But factors strongly influencing one aspect of risk do not necessarily influence the

other. Consideration of similarities and differences in effects of possible antecedents provides useful information concerning the acquisition of orientations to health-relevant risks.

For both areas of risk-taking, older children are more willing than younger ones to chance minor hazards to health and safety; this is in line with developmental expectations. General stereotypes concerning sex-role development would also suggest boys, not girls, as risk takers. This proves to be clearly the case for risks of injury, but whether the child is a boy or girl is almost irrelevant to whether simple hygienic conventions are neglected. Parental socioeconomic status does significantly influence both children's taking risks of injury and children's taking health maintenance risks--but the latter considerably more than the former. The possibility of parental observation and supervision is much greater in the case of health maintenance than in the case of playtime activity; for the latter, the child's peers may be the ones who exert pressure to bring risk performance in line with age- and sex-role expectations. Supporting this is evidence that, though sibling structure has almost no utility in predicting whether the child neglects health-maintenance routines, children with many siblings (especially older siblings) are much more likely to take risks at play than are children lacking many brothers or sisters. Serving both as role models and enforcers, older siblings and perhaps other children as well command the playing field. Parents, on the other hand, hold sway at home.

In the growing child's expected progress toward adult roles, parents obviously play a critical part. In risk-taking, as in other areas, it is likely that parents serve as role models and that by sanctions and encouragement they directly reinforce action. But such contributions to health-relevant risk taking patterns of their children also reflect two different aspects of parents' psychosocial orientations: general social values that the parent holds for the child, and more situation-specific standards for the child's functioning in circumstances that might place health at risk. Children's reports of taking chances with illness or injury do show the influence of both aspects of parental orientations, but there are differences in the chain of effects. Mothers' standards for risks in health maintenance areas reflect family position in the socioeconomic structure, perhaps implying consideration of potential differential economic consequences of illness. And these situation-specific norms of the mother significantly influence the child's own standards and, as a consequence, affect whether the child does or does not follow health-maintenance routines. For the other area of risk, chancing injury while at play, these specific maternal standards exercise little influence. Despite their being unrelated to standards concerned with health maintenance, parents' general values for their children (that is, broad goals that clearly

reflect parental characteristics and expectations linked to the child's functioning in age and sex roles) also have a considerable influence on risk-taking behavior reported by children. The influence of these maternal values is contingent on the child's age; the impact of general parental goals increases markedly as children grow older; parental values affect both areas of health-relevant risk in this manner.

These contrasts between effects of mothers' specifically health-relevant standards and their more general social values suggest that in the transmission of orientations from parent to child two different factors may be at work, one to a considerable degree situation-specific, the other much more dependent on the child's cognitive interpretations: (a) Parental control over particular situations, and the consequent possibility for direct parental reinforcement, would contribute to children's acquisition of maternal normative standards pertaining to specific health risks. But one would not expect these standards to be generalized to other risks unless the child detected underlying situational similarities. (b) The child's acceptance of parental values has the potential for much more general application to diverse areas of action. But this acceptance may require the child's playing a more active role in his own socialization process, and it may proceed at a different rate from the acquisition of situation-specific norms, a rate dependent on cognitively mediated factors. The child, responding perhaps to subtler clues of parental perspectives, must construct generalized rules largely consonant with parental values and applicable to a wide range of social behavior including those that may involve risk taking; this process of induction shows orderly age-related changes that parallel cognitive growth.

Annual Report for 1977

Laboratory of Brain Evolution and Behavior

National Institute of Mental Health

Paul D. MacLean, M.D., Chief

The human forebrain owes its remarkable triune development to the progressive evolution of three basic formations that can be characterized as reptilian, paleomammalian, and neomammalian. In reptiles the largest conducting system of the forebrain converges on a foundation of large basal ganglia. On the basis of anatomical and histochemical findings it is now recognized that corresponding structures provide the foundation of the forebrain in birds and mammals. In mammals these structures are collectively referred to as the striatal complex. One hundred and fifty years of experimentation have revealed disappointingly little information about specific functions of the striatal complex; it has remained one of the large "silent areas" of the brain. When beginning to plan for this Laboratory 20 years ago, a primary purpose was to use new behavioral approaches in investigating the functions of the striatal complex. In the meantime, general interest in this phylogenetically ancient part of the brain has increased because, in addition to acetylcholine and serotonin, its largest subdivisions have been found to be rich in dopamine and in opiate receptors.

A major research effort of the Laboratory has been to test the hypothesis that the striatal complex plays a basic role in the organized expression of naturally occurring behavior required for the survival of the individual and the species. During the past year, continued comparative studies on lizards and squirrel monkeys have provided additional evidence in support of this hypothesis by showing that destruction of certain projections of the striatal complex results in an elimination or fragmentation of species-typical display behavior. Another kind of evidence in support of the hypothesis has been obtained in experiments on hamsters in which thermal destruction of the developing neocortex was produced on the day after birth. These experiments were suggested by the remarkable findings of Laqueur *et al* that rats in which the matrix cells forming the neocortex and part of the limbic cortex were destroyed by a carcinogen, seemed to mature normally and were capable of breeding. The present experiments on hamsters not only confirmed these findings, but also showed through ethological observations that there was a retention of other naturally occurring behavior typical of hamsters. In key animals there was absence of the neocortex along with a failure of development of the neothalamus and the pyramidal tracts. It now remains to be shown how much species-typical behavior will remain after additional destruction of parts of the limbic system (paleomammalian brain). Parenthetically, Laqueur *et al* demonstrated that their micrencephalic rats were almost indistinguishable from normal animals in their performance of a wide variety of psychological tests. Based on the combined observations on micrencephalic rats and hamsters, it would appear that Lashley, when expounding the law of mass action with

respect to the neocortex, did not give sufficient attention to the two older evolutionary formations of the brain! Perhaps the work on micrencephalic animals has a message for those dealing with retarded children.

During the past year another lay-oriented book on the brain has singled out the work of our Laboratory on the two older evolutionary formations of the brain. With the growing awareness of the reptilian roots of the mammalian brain, there seems to be at the same time some confusion about the specific ancestors. Some people seem to be under the false impression that we can trace our lineage to the serpent, while others would say we are of dinosaur stock. Since a similar confusion seems to be common among neuroscientists, it may be worth while to insert a word about the unfamiliar mammal-like reptiles that are presumed to be the antecedents of mammals. During Permian and middle Triassic times these mammal-like creatures covered the face of the earth, and their remains are found on every continent. There were two main varieties: carnivores and herbivores. Some of the carnivores are believed to have resembled dogs and wolves. In late Triassic times the mammal-like reptiles mysteriously became extinct. What were these creatures like? Did they lay eggs? Did they care for their young? Or, like the offspring of the giant Komodo lizard, did the young have to escape to the trees in order to avoid being cannibalized? These and other important questions about the sense of hearing (essential for maternal-offspring cohesiveness among mammals), the disappearance of the pineal opening, and so forth, might be partially answered if more support could be given to paleontological research. Robert Broom once estimated that there are the remains of 800 billion mammal-like reptiles in the Karroo beds of South Africa.

Because of the several biochemical distinctions of the striatal complex, neuropharmacological investigations have the potentiality of shedding light on its functions. During the past year pilot experiments were conducted in regard to the question of whether or not the chronic administration of dopamine and of opiate blockers would affect the unfolding of species-typical behavior in developing animals. In other words, would such treatment affect the way in which they "read their environment." Dogs were chosen for this work because they not only show species-typical behavior, but also breed-typical behavior. Pimozide and naltrexone were used, respectively, for blocking dopamine and opiate receptors. Half of each litter of beagles served as control subjects. The findings on the pimozide-treated animals were of particular interest because of deficits in several forms of species-typical behavior, e.g., responses to handlers, allelomimetic and hunting behavior. Naltrexone-treated animals showed a number of aberrations in sexual behavior. A subsequent ethological study of the copulatory behavior of naltrexone-treated male hamsters also revealed changes in behavioral patterns. The results of the foregoing experiments illustrate that systematic ethological studies may uncover abnormalities that would not show up in conventional psychological testing.

Meynert, the famous Viennese physician, emphasized that progress in the neurological sciences requires joint effort in physiology and anatomy. This past year the Laboratory has been fortunate in having the full-time

collaboration of a comparative neuroanatomist. It has been shown in the anatomical studies, for example, that the olfactory projections overlap parts of the insula known to be involved in gustatory function. This discovery is relevant to our recent electrophysiological demonstration in squirrel monkeys of units in the limen insula, claustrum, and anterior amygdala that appear to discharge in connection with respiration and that can be entrained by vagal volleys. The combined anatomical and physiological information is pertinent to an understanding of reciprocal mechanisms underlying smelling, tasting, and swallowing. The possible significance of a similar kind of "respiratory" units found in the putamen is not apparent, but their discovery is of great interest in the light of several psychological functions known to be affected by respiration.

The Unit for Research on Behavioral Systems (URBS) is now into the second year of a six-year investigation intended to identify genetic, behavioral, and environmental factors that may contribute either to the disintegration or the enhancement of animal societies undergoing overpopulation. In one study on rats, the major purpose is to learn whether or not computer-controlled cooperation activity in an enriched environment will allow a higher degree of tolerance for overcrowding than is evident in a control group. The study thus focuses on prophylactic measures for crowded conditions. A second study on mice, on the contrary, is primarily analytical in that the major concern is to identify factors accounting for the failure of seemingly healthy young animals to develop procreational behavior when living under overcrowded conditions.

In both of the foregoing studies, special recording equipment will be used for detecting unusual changes in vocal communication. In the evolution of birds and mammals, vocalization and the necessary counterpart of hearing, become of the utmost importance in maintaining parent-offspring cohesiveness. In the mammal-like reptiles, the antecedents of mammals (see above), it is not known to what extent, if any, vocalization and hearing were involved in social communication. If they were like most living reptiles, they may have shown no care of the young and may even have cannibalized them. There are clear indications, however, that in the mammal-like reptiles two small articular bones of the jaw were destined to become part of the inner ear. It was as though there was an anticipation of the eventual necessity of maternal-offspring vocal communication among mammals. How such slow, subtle changes came about through the survival of the fittest remains for a new generation of Neo-Darwinists to explain.

A further computer-assisted mathematical analysis of an original mouse study in which the overcrowded population lost the capacity for reproduction has provided a model with provocative implications for contemporaneous human societies. The mouse population could be divided into two cohorts by using the estimated age of female loss of fertility as the dividing criterion. The course of population changes could then be described by a differential equation applicable to predator-prey populations. In the mouse population the older members would represent the predators insofar as they consumed social space that would otherwise belong to the young members. Perhaps there is a message here with respect to our burgeoning

population and an increasing number of older people who are gobbling up social space that would otherwise be available to their progeny. What the equation cannot express is that young and old are one and the same!

Annual Report of the Laboratory of Cerebral Metabolism
National Institute of Mental Health
Louis Sokoloff, Chief
July 1, 1976 through September 30, 1977

The two Sections of the Laboratory of Cerebral Metabolism did not initiate any new projects this year but consolidated and extended their research activities in ongoing projects. Both Sections have benefitted from the opportunity to utilize guest workers, visiting fellows, and visiting scientists who in many cases were supported by outside funds and served to augment their staffs and allow broader lines of investigation.

Section on Developmental Neurochemistry
Louis Sokoloff, Chief

This Section has continued its studies on the development and application of methods for studying the local cerebral circulation and metabolism and on the energy metabolism of the developing and mature brain in health and disease.

The [^{14} C]deoxyglucose method for the measurement of local cerebral glucose utilization which was first developed for use in the rat has now been developed further for application to monkeys and is now fully operational in both species. Experiments are now in progress to make it applicable in the dog, and then it will be used to study the postnatal maturation of the brain in the beagle dog which is very suitable for such studies because of its hardiness and commercial availability from uniform genetic strains.

The method has been used quantitatively in a variety of studies in the rat. Normal values for glucose consumption have been determined in over 30 structural and functional units of the conscious rat brain. The effects of thiopental anesthesia have been studied and been found to reduce cerebral glucose utilization diffusely throughout the brain, particularly in gray matter. These studies have been completed and published. The studies on the effects of γ -butyrolactone, an agent that produces a hypnotic, trance-like state, have also been completed and published. It markedly depresses cerebral glucose consumption throughout the brain. The effects of this drug are fully reversible, and it might possibly be useful as a substitute for hypothermia in surgery. The hydrolytic product of this compound, γ -hydroxybutyric acid, is normally present in brain. Its function, source, and metabolism are unknown. Studies are under way to determine the pathways of its synthesis and degradation, and the mechanisms that regulate its level in brain. An enzyme has been isolated and partially purified from brain, and its properties and activities suggest that it may be involved in the synthesis of γ -hydroxybutyrate from succinic semialdehyde, an intermediate in the pathway of GABA metabolism. Studies have also been initiated to determine whether increased levels of γ -hydroxybutyrate in brain may not be responsible for the phenomenon of hibernation in spontaneously hibernating animals, such as the hamster. Preliminary results indicate a considerable increase in the activity of the γ -hydroxybutyrate: succinic semialdehyde oxidoreductase enzyme in the brains of the hamster during hibernation.

O. Sakurada (Visiting Associate from Japan) has completed his studies in collaboration with W. Klee (Laboratory of General and Comparative Biochemistry) on the effects of acute morphine administration, morphine addiction, and morphine-withdrawal on local cerebral glucose utilization in the rat. The results have demonstrated that a number of cerebral structures are affected, generally by the reduction of glucose consumption, in morphine addiction and that a few of them, most notably the habenula, show a reversal of this depression on naloxone-induced withdrawal.

M. Shinohara (Visiting Fellow from Japan) has also completed her studies on the effects of LSD which also reduces cerebral glucose consumption selectively in a number of cerebral structures.

L. Wechsler (summer student) and H. Savaki (Visiting Fellow from Greece) have carried out studies of the effects of the dopamine-agonist, amphetamine. It was found that this drug, which in toxic doses produces a schizophrenia-like state, markedly increases glucose consumption in the substantia nigra, caudate nucleus, and subthalamic nucleus, all components of the extrapyramidal motor system.

The [^{14}C]deoxyglucose method employs an autoradiographic technique for the measurement of the tissue radioactivity. Even without quantification the autoradiographs, which are really pictorial representations of the relative rates of local cerebral glucose utilization, can be used to map the areas of brain that are metabolically and functionally activated or depressed. C. Kennedy (Guest Worker, Georgetown University) has used the technique to map the functional visual pathways in the adult and newborn monkey. He has been able to pinpoint the exact locus, size, and shape of the visual cortical loci of the blind-spots of the visual fields and has been able to visualize the full structural pattern, distribution, and extent of the ocular dominance columns of Hubel and Wiesel. These columns have been found to be present at birth in the newborn monkey and to exhibit the capacity to be modified in early life by visual experience; patching of one eye for 3 months causes the striate cortex to be modified to represent only the function of the functional eye.

E. Kaufman and T. Nelson (Guest Worker, Georgetown University) have continued studies on the physiological implications of ketone body utilization by brain. The possibility that some types of metabolic coma, such as diabetic coma, might be caused by the forced excessive utilization of ketone bodies by brain because of the severe ketosis, is still under study. Preliminary results indicate that ketosis may lead to a reduction in cyclic GMP in the brain. Since this compound appears to be the second messenger for the action of the neurotransmitter, acetylcholine, in the nervous system, it may be that ketosis may lead to coma by a tendency to block cholinergic functions in the brain.

Section on Myelin Chemistry
Marian W. Kies, Chief

Autoimmune damage by some constituent of CNS tissue is the most logical explanation for the cause of the human disease multiple sclerosis (MS). Although myelin basic protein (BP) has not been directly implicated in the development of MS, it is the only CNS antigen known to induce an experimental

autoimmune reaction. As such it is the only tool available for studying the possible mechanisms involved in the induction of MS.

The efforts of the Section on Myelin Chemistry have been devoted to studying many aspects of the chemical and immunologic activity of this protein: its ability to induce allergic encephalomyelitis (EAE) in various species; its tissue localization; its ability to prevent or suppress EAE; the effect of conformation on its activity; and the contribution of impurities to its observed immune reactivity.

Last year Dr. Martenson and Dr. Nomura reported the essential requirements for induction of EAE in the rat: Peptide (72-84) of guinea pig BP is the shortest fragment yet found to be active in this species. Dr. Nomura has continued his search for proteolytic enzymes capable of splitting specific bonds which may be used to prepare even smaller encephalitogenic peptides.

Dr. Martenson is currently examining the possible secondary structure of this peptide with molecular models. With synthetic peptides provided by Dr. Janis Young of the University of California, it is hoped to be able to define the specific conformation which is essential for the encephalitogenic activity of this region.

Another major interest of the Section has been the mechanism of prevention or suppression of EAE in guinea pigs. Earlier studies published by this laboratory have shown that BP in a nonencephalitogenic form (emulsified without mycobacteria) is capable of interfering with EAE induction by BP in an encephalitogenic form (emulsified with mycobacteria). In a study of the protective ability of various fragments of BP, Dr. Driscoll concluded that protection was afforded only by fragments which contained the intact encephalitogenic site, residues 113-121. During the past year we were able to compare two very similar fragments: (111-169) and (116-169); the former was encephalitogenic because it contained all of the active site (residues 113-121) and the latter was non-encephalitogenic because three essential residues were missing (113-115). As expected, 111-169 afforded complete protection and 116-169 was completely nonprotective. These peptides and others being used for critical studies of antibody specificity have been prepared by Drs. Martenson and Nomura. Their characterization requires a great deal of careful analytical work on the part of Mrs. Deibler.

The members of the section are convinced now that the two activities (induction and protection) are interrelated although they have not yet been able to explain the underlying mechanisms. In other experimental situations with other antigens, "effector" and "suppressor" cells have been proposed as mediators of the two opposing activities. In their in vivo experiments they have not been able to demonstrate the existence of "suppressor" cells but plan to extend the study of the mechanisms of induction and suppression with in vitro experiments."

In collaboration with Drs. Henry Webster and Nancy Sternberger of NINCDS studies have been carried out on the localization of BP in CNS tissue of neonatal rats. Progress has been made in developing new fixatives which permit better immunocytochemical visualization of the antigen in tissue sections and

in isolated myelin fractions. Antisera used for these studies were prepared by Mr. Stream and their immunologic characterization carried out by Mrs. Bacon.

Techniques for detection and identification of impurities in BP preparations are especially critical to studies of immunologic reactivity. Mrs. Deibler has just completed isolation and identification of a particularly troublesome impurity in bovine brain BP which we had recognized only as a band on acrylamide gels and which accompanied BP through many purification steps. By careful fractionation, she was able to obtain BP essentially free of the contaminant and enough of the contaminant essentially free of BP to permit its identification as the α chain of bovine hemoglobin. There is indirect proof that its presence may account for some of the immunologic activity reported for bovine BP by other investigators.

Annual Report of the
Laboratory of General and Comparative Biochemistry
National Institute of Mental Health
Giulio L. Cantoni, Chief

Fiscal year 1977 has been a very good year for the Laboratory of General and Comparative Biochemistry. Considerable progress has been made in a number of projects; however, budgetary restriction and especially personnel ceilings in all categories continue to limit severely the productivity of the four senior, permanent members of the staff. These investigators are all fully developed, independent scientists, at the peak of their productive scientific career. All could utilize much larger manpower resources to explore and pursue interesting and promising new ideas and/or to follow up on discoveries and developments generated by their own research. Conceivably, many of these ideas might lead to significant advances in the prevention, diagnosis or treatment of disease. Instead of being able to do this, we are all obliged to restrict our field of operation and try to select a very limited number of research projects that seem likely to yield results. This kind of selection process is the most hazardous and difficult task facing a scientist and even the very best investigator is often unable, a priori, to decide which one of two or more equally promising ideas or discoveries is likely to lead to a significant advance. The limitations and lack of flexibility in the size of research group available to our senior investigators thus imposes an unnecessary element of risk in the optimum utilization of our research capabilities.

To overcome, at least partially, these limitations we have been utilizing, whenever possible, the collaboration of colleagues both at NIH and elsewhere; but even this type of collaboration with colleagues not at NIH is hampered by budgetary travel restrictions. The same restrictions also affect the flow and exchange of scientific information to our scientists since attendance at scientific meetings is limited. It should be pointed out that the restrictions with respect to all kinds of travel affect considerably more the scientists working in the intramural program of NIMH or NIH than our colleagues in universities as the roster of attendance to any meeting will clearly demonstrate. On the bright side of the budget picture, it may be pointed out the size of our budget for other objects has been adequate to satisfy our needs for equipment and supplies.

Drs. Cantoni, Uhlendorf, Scarpa and Chen continue to study muscle differentiation in vitro. The basic biochemistry of the differentiation process is very fundamental in the normal

development of the various cell types of the brain and also of many other tissues. They have chosen as a model the growth and differentiation of embryonic skeletal muscle cells in culture. In the course of experiments designed to investigate the role of the cell membranes in the complex series of events that underly the differentiation of mononucleated myoblasts into multinucleated myofibers, they discovered that Concanavalin A and a variety of other plant lectins have an important effect on the differentiation of myoblasts in tissue culture.

It will be remembered that differentiation of myoblasts is associated with a variety of physiological, histological and biochemical changes that are thought to represent the differential reading of the cellular genome. The biochemical changes that have been investigated rather extensively consist of a) inhibition of DNA synthesis that is correlated with the arrest of cellular division; and b) the synthesis of muscle specific proteins such as creatine kinase, myosin, actin and other proteins important in muscle function. Heretofore, these different events had not been dissociated in time and thus it had not been possible to order the changes seen in differentiating myoblast into early and late events.

Concanavalin A and other lectins were found to inhibit the physiological and histological changes, such as muscle fusion and appearance of cross striation, and also to inhibit the synthesis of creatine kinase and other muscle specific proteins. On the other hand, these lectins do not affect the cessation of growth and the inhibition of DNA synthesis that, as noted above, is another parameter of differentiation of myoblasts in vitro. Moreover, it was discovered that the effect of Concanavalin A is strictly dependent on the time of its administration. It should be pointed out that the differentiation of myoblast in culture is a slow process that is observed after seven days of culture in a "fusion permissive medium," the nature of which has been determined in this laboratory by earlier studies. The end point of the experiment, i.e., the appearance of multinucleated fibers is scored at day seven. Administration of Concanavalin A during the first four days of the experiment results in the inhibition of differentiation discussed above, whereas its administration later in the experiment (days 5 to 7) is without effect. The conclusion from this important discovery is that it is now possible to divide the course of differentiation into two phases. During the first four days the cells respond to the exogenous stimulus that triggers differentiation (the change from growth medium to fusion permissive medium) by a number of changes, the molecular nature of which is unknown, but that somehow result in inhibition of growth, arrest of DNA synthesis and development of resistance to the inhibitory effects of plant lectins. During the second phase of the experiment, the changes that have taken

place in the period of commitment, as the first phase may be tentatively labelled, are expressed and synthesis of muscle specific proteins as well as physiological and histological changes, such as development of cellular receptor for acetylcholine cross striation and formation of multinucleated myofibers can be measured. The nature of biochemical changes taking place during the "commitment phase" and the molecular mechanisms whereby an exogenous signal brings about changes in the expression of cellular genome are central and most interesting questions and our results will permit further exploration of this problem.

Drs. Cantoni and Chiang, with the collaboration of Henry Richards, have continued to study the metabolism of S-adenosylmethionine (AdoMet) and S-adenosylhomocysteine (AdoHcy) with the aid of chemically synthesized analogues of AdoHcy. It may be remembered that AdoMet functions as the methyl donor in hundreds of reactions of great physiological significance. These involve, in all phyla, methylation of macromolecules, such as DNA, RNA, proteins and polysaccharides and, in a variety of different phyla, methylation of a great many different compounds leading to the synthesis of metabolites that are important in the physiology of many different types of cells and organisms, such as creatine, carnitine, epinephrine and many other methylated catecholamines and indolamines, phospholipids and related compounds, such as acetylcholine, methylhistamine, alkaloids and antibiotics. AdoHcy is the product of each methyltransfer reaction in which AdoMet is the methyl donor and is a competitive inhibitor of practically all the enzymes that utilize AdoMet as a methyl donor.

A question that has remained unanswered for 25 years, ever since Cantoni discovered AdoMet, is the nature of the regulatory mechanism that govern the utilization of AdoMet in the cell. In order to begin to approach this question, it was decided several years ago to synthesize a large number of analogues to AdoMet and AdoHcy in the hope of finding compounds capable of inhibiting one or more of the several reactions involving AdoMet and AdoHcy. In the course of the last year, it has been found that 3-deaza-adenosine (as well as a number of other synthetic analogues) are powerful inhibitors of AdoHcy hydrolase in vitro. In the extension of these studies to whole cells, it was found that in vivo administration of 3-deazaadenosine to a variety of cellular preparations resulted in accumulation of AdoHcy, 3-deazaadenosine Hcy and in some cases of AdoMet.

This discovery is very significant and of great interest because it will permit the study of the physiological consequences of interference with the metabolism of AdoMet. Along these lines, it has already been found, in collaboration with John Bader, that the replication and growth of Rous sarcoma virus in chick embryo fibroblasts is prevented by administration of 3-deazaadenosine;

moreover, the malignant transformation induced by the oncogenic virus is reversed by treatment with 3-deazaadenosine. Further work on the mechanism of this significant effect is in progress.

The Section on Alkaloid Biosynthesis has continued to study the metabolism of sulfur amino acids in plants and in man. Studies of the work on sulfur amino acid metabolism in plants has been concentrated chiefly upon Lemna perpusilla, a higher plant which by virtue of its aquatic habit, its ability to grow rapidly, axenically, and vegetatively, and its capacity to take up amino acids from the medium is ideal for such studies. The work initiated during Dr. Macnicol's visit has been completed. The results demonstrate unequivocally that in Lemna, as in Chlorella, the major pathway for homocysteine biosynthesis is transsulfuration in which the intermediate, cystathionine, plays a key role. In contrast with the situation in Chlorella, however, our present results indicate that 5-10% of homocysteine biosynthesis in Lemna does occur by direct sulphydration.

By use of a special apparatus designed and built in the laboratory, Dr. Mudd and his collaborators have been able to grow Lemna continuously at extremely low, but constant concentrations of sulfate in the growth medium. A decrease of 3000-fold in the usual sulfate concentration did not lead to a significant change in growth rate. Under these conditions cellular sulfate decreased almost 40-fold, but, remarkably, the total net conversion of inorganic forms of sulfur to organic sulfur was essentially unperturbed. This observation suggests a high degree of regulation at some step between sulfate uptake and cysteine formation. An understanding of this regulation might be very important in increasing overall production by plants of their chief organic sulfur constituents, methionine and cyst(e)ine.

These results have been made possible, in part, because Dr. Mudd and his collaborators have continuously improved the analytical methods for dealing with sulfur compounds. During the last year they have found that S-methylmethionine accounts for a major hitherto unidentified, soluble sulfur-containing compound of Lemna. Methods for quantitating S-adenosylmethionine at the very low concentrations at which it is present in Lemna have also been developed. It is now possible to specify the chemical forms in which at least 90% of the total sulfur of Lemna occurs. This rather complete accounting is expected to strengthen the rigor with which a number of projected experiments can be interpreted.

Dr. Mudd and his collaborators have also carried out preliminary experiments bearing upon the regulation of methionine biosynthesis. (a) In other laboratories, it has been observed with several plants that lysine and threonine, in combination, exert a growth inhibiting effect which is not produced by either amino acid alone. The growth inhibition may be relieved by methionine. Each of these three compounds is an end product formed from aspartate. It is

suggested that the lysine plus threonine combination leads to a depression of homoserine formation with resultant methionine starvation. This effect has been demonstrated very convincingly with Lemna perpusilla. (b) Experiments detailing the flux of sulfur in plants grown in the presence of methionine have been started. Results are not yet available, but it appears already that it is possible to increase very significantly the total methionine content of plants without severely inhibiting growth, and to bring about dramatic rises in the concentration of S-adenosylmethionine, an effector in many regulatory phenomenon in other biological systems.

All these results have indicated the value of Lemna as an experimental system in which a great variety of questions concerning amino acid biosynthesis and its regulation can be asked. Accordingly, this year a major effort has been made to define optimal conditions for growth of this plant, and the limits within which a variety of physical and chemical factors must be controlled to avoid inadvertant changes in growth. To facilitate eventual ^{14}C labelling experiments, Dr. Mudd and his collaborators have also defined conditions under which CO_2 , the ordinary carbon source for growth, may be replaced with sucrose. This work should permit standardization of conditions for a great variety of experiments in the years to come.

Finally, although it has this year been possible to devote less time to the project on human inborn errors of metabolims involving sulfur containing amino acids, a project has been initiated to evaluate the possibility that heterozygotes for homocystinuria due to cystathionine synthase deficiency may be at significant risk for early coronary artery disease. From available data, it appears just possible that this factor could account for a major portion of the "unexplained" coronary artery disease in the U.S. population. Whether or not this working hypothesis is verified, it is expected that the methodology used in this study will facilitate the evaluation of analogous hypotheses which may be raised with respect to a number of other inborn errors of metabolism.

Carl Merrill continued to explore the expression of bacterial virus genes in eukaryotic cells. Work on expression of genes from lambda virus regulating galactose metabolism in galactosemic human cells has been impeded by the lack of a selection system for cells expressing the bacterial galactose operon genes. Enzyme activity can be demonstrated after infection, but without a selection system thorough biochemical and genetic analysis cannot be accomplished. On the other hand, excellent selection systems for mammalian cells deficient in the purine phosphoribosyltransferase enzymes exist. Micro assays for these enzymes have been developed and bacterial viruses and plasmids containing the enzyme genes have been isolated and are being tested. This system may thus be more useful than that derived from galactosemic

human cells for the study of the factors that regulates the expression of foreign genes derived from bacterial virus in eukaryotic cells.

Dr. Klee and his colleagues have, over the past year, continued their studies on the relationships between opiate receptors, the endogenous opioid peptides and adenylate cyclase both in brain and in the neuroblastoma x glioma hybrid cell system. The hybrid cells, which are richly endowed with opiate receptors, are once again proving to be a valuable tool for biochemical work since they are relatively deficient in enzymes which degrade opioid peptides. It has therefore been possible to study the effects of these peptides, subsequent to receptor binding, without serious complications due to their destruction by the adventitious peptidases which are found in great abundance in brain. Dr. Klee has found that, contrary to results obtained with brain in vivo and in vitro, the pentapeptide methionine enkephalin is a more potent opioid (as measured by the receptor mediated inhibition of adenylate cyclase) than are any of the larger peptide fragments of β -lipotropin (β -LPH) including some, such as β -endorphin (β -LPH 61-91), which in in vivo seem much more potent than is enkephalin. Since enkephalin is very rapidly inactivated in brain, these experiments raise the possibility that enkephalin may, in fact, be the physiologically most important opioid peptide and that the larger peptides may represent a more stable, depot, form from which enkephalin is released upon demand.

In collaboration with Lampert and Nirenberg, Dr. Klee has found that the opioid peptides share with morphine and its congeners the property of inducing tolerance and dependence in neuroblastoma x glioma hybrid cells. This result is consistent with biochemical model for tolerance and dependence proposed by Klee and Nirenberg and is in accord with animal studies from other laboratories. In studies carried out in collaboration with Jacquet and Rice, it was found that some effects of morphine, in vivo are not mediated by the usual opiate receptors. The hyperresponsivity to audio-visual stimuli seen after microinjection of morphine into the periaqueductal gray or the midbrain reticular formation was found to be elicited by the (+) (unnatural) as well as the (-) (natural) enantiomers of morphine. Analgesia, on the other hand, is observed only upon administration of (-) morphine. These results suggest that the hyperexcitability seen in the morphine abstinence syndrome may, in part, be the result of residual stimulation of this new and presumably different class of receptor. Since the opioid peptides were found not to cause such excitability, it is conceivable that withdrawal following dependence upon opioid peptides may be much milder than is the morphine abstinence syndrome, and on this basis it may be anticipated that opioid peptides may find some clinical application.

ANNUAL REPORT OF THE LABORATORY OF NEUROBIOLOGY
National Institute of Mental Health
July 1, 1976 to September 30, 1977

Ichiji Tasaki, M.D., Ph.D., Chief

The macromolecular structure of the nerve membrane involved in the process of excitation has been studied by Dr. Ichiji Tasaki and his collaborators, Dr. Tohru Yoshioka, Dr. Harish C. Pant and Dr. Jesse Baumgold.

Protein modifying reagents and proteolytic enzymes were applied to both crab nerves and squid giant axons. The experimental results obtained by using these methods during the past year have given us some information on which functional groups are important in nerve excitation. A large number of protein modifying reagents were applied either extracellularly to crab nerves or intracellularly in squid giant axons. Reagents which modify sulfhydryl groups, imidazole groups, and methionine groups in proteins were found to block nerve conduction under certain conditions. No nerve conduction block could be found which could be attributed to the specific modification of amino groups. This information is important in developing a site-specific probe which could be used in further analyzing the proteins involved in nerve conduction.

Although the electrophysiological effects of applying proteolytic enzymes to the inside of squid giant axons have been studied for years, most of these previous studies have taken into account the effect of the salts in the solution in which the enzymes were dissolved. In the current experiments it has been found that the time necessary for nerve conduction block due to intracellular application of these enzymes is dependent on the salt solution in which it is dissolved: when dissolved in a potassium fluoride containing solution, the time necessary to block conduction was generally longer than when the enzyme was dissolved in a solution containing a more chaotropic salt such as potassium aspartate.

In an effort at understanding the role of axonal proteins in maintaining the excitability of squid giant axons, several methods have been developed which have provided information on these proteins. These methods all depend on radioactively labelling, either *in situ* or *in vitro*, the axonal proteins using a variety of commercially available reagents. Two radioactive reagents, ¹²⁵I-Bolton-Hunter reagent and ³H-dinitrofluorobenzene were used to label squid giant axons *in situ* by introducing these reagents into an axon. It was found that electrical stimulation of the axon during the radioactive labelling increased the labelling efficiency of the reagents, resulting in a higher specific activity of the labelled axonal proteins in stimulated compared to non-stimulated axons. This increase in labelling efficiency was attributed to the increased exposure of protein sites during excitability.

The work of Dr. Robert H. Wurtz and his collaborators, Dr. Barry J. Richmond, Dr. Stuart J. Judge and Dr. Joanne E. Albano, has concentrated on the organization of visual processing and eye movements in order to understand how the brain translates sensory input into eye movement output. This year they have concentrated on one aspect of this general problem which has long fascinated those interested in the brain: how the brain deals with sensory stimulation resulting from movements initiated by the brain itself. Each saccadic movement of the eyes smears across our retina the image of the scene before us, a fact of which we are normally unaware. In last year's annual report work was summarized which indicated that visual stimuli normally present before and after each saccade obliterate or "mask" the visual smear which is always present during an eye movement. This year the physiological basis of this masking effect was investigated by recording from the primary (striate) visual cortex of the monkey, because activity in the corresponding area in man is known (from studies of patients) to be closely related to visual sensory experience.

In these physiological experiments awake monkeys were used which were trained to fixate their gaze on a point of light on a screen in front of them. Of the single neurons whose electrical activity was recorded in the visual cortex, approximately one third responded as the monkey made a rapid eye movement across a visual stimulus. However, if a stimulus was on the receptive field of the cells before and after the eye movement, the result was quite different: then the cells did not give a detectable response to the stimulus present during the eye movement. Thus the response of the cells was lost just as visual perception to the smear on the retina was lost when a clear image preceded and/or followed the saccadic eye movement in normal vision. These experiments and those reported last year answer the question of why at both the behavioral and physiological level we do not see during eye movements.

The visual pathway from the eye to the brain in both man and monkey has a second branch which extends directly from the eye to the superior colliculus lying on the roof of the midbrain. Cells in the superior colliculus are actively suppressed during eye movements and the question answered this year was whether the suppression of activity is due to the sensory information flowing into the brain during an eye movement from the eye muscles, or whether the suppression is due to a message from within the nervous system which is a corollary of the impulses sent to the muscles causing the eyes to move.

In these experiments the proprioceptive input was removed by paralyzing the eyes. The occurrence of attempted eye movements was indicated by the discharge of cells recorded from an electrode previously implanted in the motor nucleus connected to the eye muscles. The discharge of cells which showed the suppression of background activity with normal eye movements continued to show the suppression during the complete paralysis. For several cells, the suppression was observed before, during, and after the period of paralysis. Since the

suppression is present when no eye movement occurs, no signal related to the eye movement (including proprioception can be causing the suppression: instead the suppression must have its origin in that part of the nervous system related to the intention to move the eyes. This is the first time such a powerful corollary discharge has been shown to modify visual processing of cells in the visual system of mammals.

These experiments show that the brain deals with sensory stimulation during eye movements in two different ways. In the primary visual cortex, which is closely related to visual perception, cells are modified by interaction of the successive visual stimuli. In the other branch of the visual system, the superior colliculus, activity from oculomotor areas of the brain reduce the sensitivity of the colliculus cells to visual stimulation. But but rather than performing a perceptual function, this corollary discharge may eliminate spurious self-generated visual signals from reaching the oculomotor system.

Annual Report of the Laboratory of Neurochemistry
National Institute of Mental Health
July 1, 1976 - September 30, 1977

Seymour Kaufman, Chief

Our detailed analysis of the mammalian phenylalanine hydroxylase system, as well as that of closely related tyrosine and tryptophan hydroxylating systems, showed that these systems are composed of at least three essential components, the hydroxylase, dihydropteridine reductase (DHPR) and the pterin cofactor.

Based on this analysis, we were able to predict that in addition to classical phenylketonuria (PKU), which is caused by a lack of phenylalanine hydroxylase, two other variants might occur that would be caused by the deficiency of either DHPR or the pterin cofactor.

In the last two years, the initial discovery and characterization of the first of these variants, hyperphenylalaninemia (HPA) due to a lack of DHPR, was described. During this last year, the first case of hyperphenylalaninemia due to a deficiency of the pterin cofactor was discovered. This variant is also characterized by mental retardation and delayed motor development. The pathology became manifest despite early control of hyperphenylalaninemia by dietary restriction of phenylalanine intake. In this child, biochemical analysis on a biopsy liver sample showed that the levels of phenylalanine hydroxylase and DHPR were normal or high normal but the level of pterin cofactor was only 5 to 10% of normal.

Since the pterin cofactor is an essential component of brain tyrosine and tryptophan hydroxylases, we have suggested that this child's abnormal development is caused by defects in the biosynthesis of dopamine, norepinephrine and serotonin. Just as with hyperphenylalaninemia due to DHPR deficiency, we have proposed that the administration of DOPA and 5-hydroxytryptophan be used to treat this new disease.

If any treatment is to be effective, it most likely will have to be initiated as early as possible. We have shown that the deficiency of pterin cofactor can be detected in the patient's urine. This test should provide a means for the rapid diagnosis of hyperphenylalaninemia due to pterin cofactor deficiency and should thus allow an early start of therapy.

We have previously developed a quantitative *in vivo* assay for phenylalanine hydroxylase activity that is based on the fact that the conversion of ring-tritiated phenylalanine to tyrosine is accompanied by the liberation of tritiated water into the body fluids. In collaboration with Dr. J. Schulman, NICHD, we have for the first time performed this assay on a human subject. The child with cofactor deficiency was found to have between 1 and 5% of normal phenylalanine hydroxylase activity. These results agree very well with the phenylalanine hydroxylase activity that would

have been expected from the low amounts of cofactor that were detected in his liver. This assay is sensitive enough to be useful in discriminating between classical PKU, with phenylalanine hydroxylase activity of less than 1% of normal, and "atypical" PKU, with phenylalanine hydroxylase activity of 3 to 5% of normal.

As mentioned earlier, we have previously proposed that administration of DOPA and 5-hydroxytryptophan might prevent the neurological deterioration that characterizes hyperphenylalaninemia due to DHPR deficiency. Experience with this therapy during the last year has provided a new perspective for assessing its effectiveness. It is clear now that the treatment cannot reverse the neurological deterioration once it occurs. There is evidence, however, that it stops the progressive downward course that characterizes the disease. In those cases where replacement therapy was not instituted, the disease seems to be fatal. Indeed, it is being called "lethal hyperphenylalaninemia". By contrast, to date, no patient who has been treated with DOPA and 5-hydroxytryptophan has died from this disease.

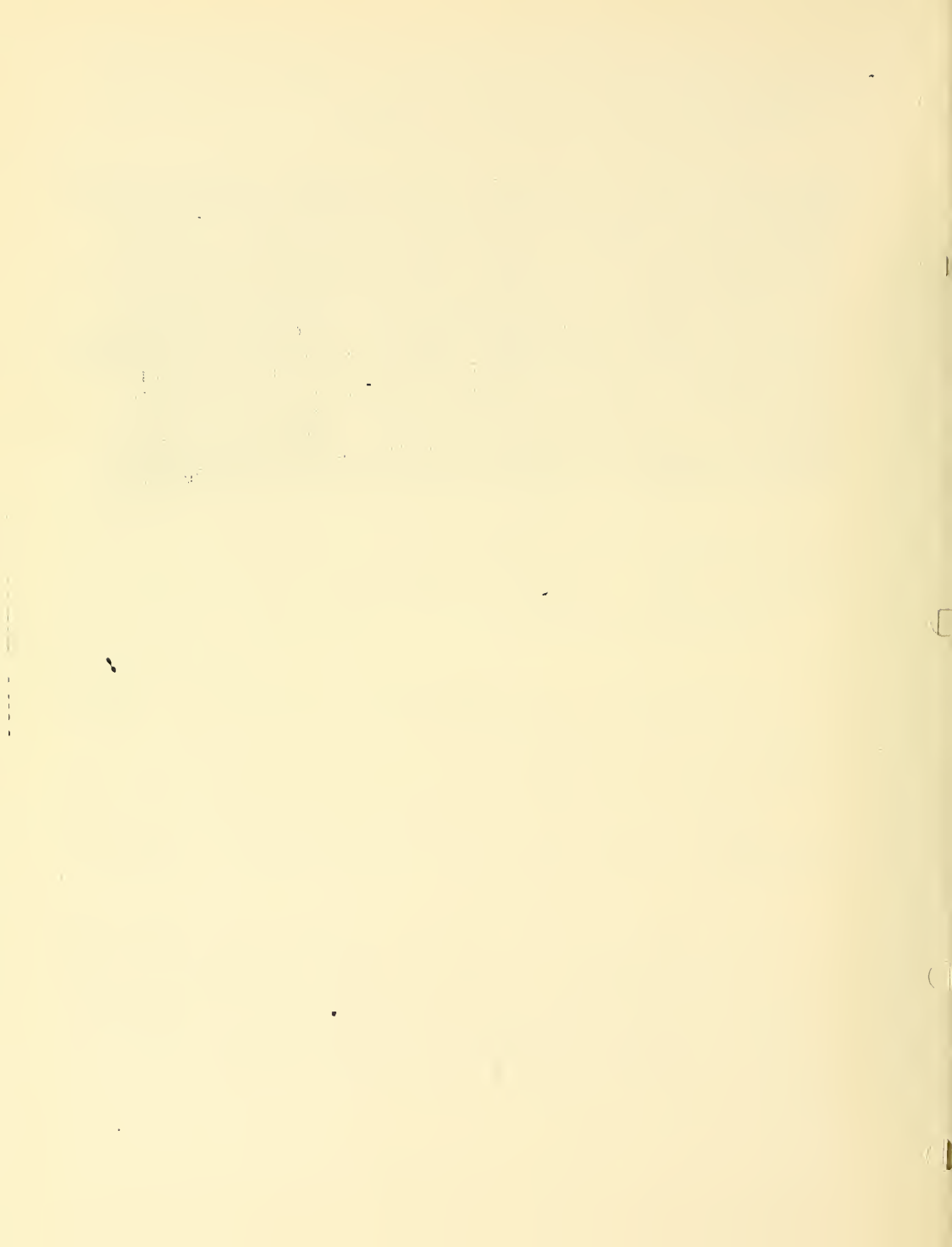
During the last year, work in the Laboratory of Neurochemistry led to the solution to a puzzle of brain metabolism. The elements of the puzzle are: a) tetrahydrobiopterin is synthesized in the brain; b) the most likely synthetic route goes via 7,8-dihydrobiopterin; c) the only enzyme known to convert 7,8-dihydrobiopterin to tetrahydrobiopterin is dihydrofolate reductase; and d) dihydrofolate reductase has been reported to be undetectable in brain. We found that the last "fact" is incorrect. Brain does have dihydrofolate reductase and the brain enzyme can synthesize tetrahydrobiopterin from the 7,8-dihydro derivative. The finding of dihydrofolate reductase in brain clarifies many heretofore mysterious aspects of brain metabolism.

A significant advance in our understanding of the mechanism of skeletal muscle hypertrophy was achieved recently. We had previously found that the earliest biochemical change in this process is a marked increase in phospholipid metabolism in the hypertrophying muscle. This result could not be interpreted, however, because the cell type in which the enhanced metabolism was occurring had not been identified. With the use of newly developed autoradiographic techniques, we were able to show that the increase in phospholipid metabolism occurs in muscle cells before it occurs in other cell types. This alteration in metabolism is, therefore, not only the earliest one detected to date, but also the earliest one that has been localized to muscle cells.

Dr. Nash and his coworkers have made an important advance in our understanding of the mechanism of integrative recombination in *E. coli*. They have detected and isolated a new enzyme, called "DNA gyrase, that play a key role in the process of integration. In the presence of magnesium ions and ATP, the enzyme converts closed circular DNA into the supertwisted DNA that is required for integrative recombination in cell-free extracts. That the activity of DNA gyrase is also essential for integrative recombination in whole cells was shown with the use of certain

antibiotics that work by inhibition of the gyrase.

In the Section on Biophysical Chemistry, Dr. Neville and his colleagues have continued to explore the process of plasma membrane receptor-mediated entry of proteins into the cytosol of cells. They have succeeded in constructing artificial hybrid molecules (i.e., molecules that do not occur in nature), between 2 protein chains, each of which has a different physiological function. One such molecule that they synthesized is a hybrid of diphtheria toxin fragment A and human placental lactogen. This hybrid was used to determine whether the interaction of lactogen with specific receptors on mammary gland cells would mediate the entry of the toxin chain into the mammary gland cell. Since the toxin is known to inhibit protein synthesis within cells, the state of intracellular protein synthesis serves as a detector of functional toxin molecules within the cell. The results indicate that the enzymatic activity of the toxin A molecule was lost within the cell. New methods are being explored to protect the enzymatic active site and to further study this important new means for delivery of physiologically active molecules to specific cells.



Annual Report of the Laboratory of Neurophysiology

National Institute of Mental Health

Edward V. Evarts, M.D., Chief

July 1, 1976 -- September 30, 1977

The projects of the Laboratory of Neurophysiology may be categorized according to three major areas of ongoing research:

I. Brain Mechanisms in Motor Control

1. Cerebral control of movement
2. Eye-head coordination in the primate: neural substrate and plasticity
3. Cortical response to sensory input as a function of steady state motor activity

II. Neurochemical Correlates of Activity in Brain and Muscle

1. Metabolic correlates of brain functional activity
2. Muscle function and metabolism

III. Functional Neuroanatomy

1. Transport mechanisms across membranes and the blood-brain barrier
2. Functional studies of the substantia nigra
3. CNS connections studied by amino acid autoradiography and horseradish peroxidase

This summary will consist of a presentation of some highlights of the findings in each of the three areas, with a statement as to the significance of these findings to the research goals of the National Institute of Mental Health.

I. Brain Mechanisms in Motor Control

Animal studies on brain mechanisms in motor control have elucidated the relation of motor cortex neuron discharge to large (20°) ballistic movements as compared to small ($\sim 1^\circ$) precisely controlled movements. It was found that neuron responsiveness to sensory input is relatively enhanced during accurate positioning and controlled small movement, but reduced before and during ballistic movements. Taken together, these findings demonstrate that the transcortical servo-loop is continuously active and powerful during precisely guided fine movements and posture. The intense and prolonged discharge of precentral neurons during these fine movements must partly reflect the central motor program and partly reflect sensory feedback in a closed loop. The relative contribution of the two inputs to the motor cortex output remains to be fractionated. The relative lack of sensory feedback to motor cortex just before and

during a ballistic movement appears to depend on the nature of the central preprogram and on the specific parameters of the movement itself.

A second phase of the sub-human primate work on brain mechanisms in motor control has focused on elucidation of neural mechanisms underlying plasticity in the nervous system. In this project we have examined the effects of manipulating the visual input normally associated with head movements on the vestibulo-ocular reflex (VOR). Normally, monkeys (like man) rely on the VOR to maintain a stationary retinal image during head turns. However, our previous studies have shown that this system is plastic and subject to a visually mediated optimization process which assures that the operation of the VOR maintains maximal stability of the retinal image during head turns; thus, when animals wear telescopic spectacles the VOR adapts to the new conditions by increasing or decreasing gain (i.e., sensitivity) depending on the power of the lenses being worn by the animal.

Experimental objectives in work on the VOR were twofold: 1. Behavioral studies have attempted to define quantitatively the dynamic characteristics of the VOR after it has been modified through prolonged optical reversal, magnification or reduction of the visual input normally associated with head movements. 2. Chronic single unit recording techniques were initiated with the object of locating the site of the neural modifications which underlie these adaptive changes in the VOR.

It has been found that animals that have worn telescopic spectacles show adaptive changes in the gain of their VOR. The normal rhesus monkey has a VOR gain of close to unity, and after x2.0 lenses values up to 1.85 are recorded, while after x0.5 lenses values as low as 0.6 are found. These changes take 2 to 5 days to reach asymptote, and recovery following removal of the spectacles takes a similar period of time. These changes follow an exponential time course so that the rate of change of gain is proportional to the error, i.e., the difference between the actual VOR and that called for by the power of the lenses. There is no "progressive learning effect," i.e., with subsequent repetitions of the spectacle experience, the time required to adapt does not shorten. Prolonged head immobilization after prior adaptation considerably delays recovery back to normal.

These effects of telescopic spectacle experience on the gain of the compensatory eye movements coupled to head turns clearly indicate that the primate vestibulo-ocular system is both plastic and adaptive, seeking always to optimize retinal image stability. The relative simplicity of this system, its easy quantification, and our ability to induce modifications in its performance without apparent pathological side effects, make it a powerful new substrate for studying plastic mechanisms in brain function. Prolonged optical reversal of vision results in both gain and phase changes in the VOR, but the dynamics of the "reversed" responses are very abnormal, suggesting that they are subserved by a different neural substrate from that mediating pure gain changes. Thus, there seems to be more than one modifiable element in this system.

Single unit data have been gathered from the vestibular nerve and flocculus of three normal monkeys and four gain-changed animals, and recordings are still under way. The preliminary indications are that the modifications underlying

the adaptive capability of the VOR do not occur in either the labyrinth or the flocculus. Indeed, such changes as are seen in the flocculus suggest rather that this structure receives a signal which has already been partly modified.

The single unit recordings are being undertaken to establish the exact locus of the modifiable elements in the VOR. A great deal is known about the anatomy and physiology of the VOR, which represents one of the best examples of adaptive plasticity currently available in the primate brain. Once we know exactly where the modifiable elements are located, a whole new area of research related to aspects of motor learning will be opened up.

In addition to the above-summarized work on monkeys, human studies on brain mechanisms in motor control have been carried out to obtain a clearer delineation between the reflex and the intended components of arm movements in normal subjects and patients with psychomotor disorders. To this end we have used a paradigm in which a given direction of intended arm movement is triggered by either of two possible directions of limb displacement, with one direction stretching and reflexly exciting the biceps muscle and the other direction shortening and reflexly inhibiting the biceps musculature which was to become active with the intended response. When the triggering perturbation "unloaded" and inhibited the biceps muscle which the subject intended to activate the intended discharge was superimposed on the silence of reflex inhibition and the time of onset of this intended discharge could be accurately identified.

Using this paradigm, we find that the response to a kinesthetic stimulus involving muscle stretch consists of: 1) a spinal reflex; 2) a longer latency reflex mediated by supraspinal circuits; 3) an intended response which is more rapid than the intended response to a stimulus which does not involve displacement of the responding limb. Studies in patients with parkinsonism are aimed at seeing how these components of motor response can be used to assess clinical status and aid in the regulation of drug treatment. Systematic studies have been carried out in 20 patients in collaboration with the clinical staff of NINCDS. Results are still too preliminary to serve as a basis for conclusions, but give promise of ultimately providing useful tools for patient assessment and management. Preliminary results have also been obtained in patients with Huntington's disease.

II. Neurochemical Correlates of Activity in Brain and Muscle

Two projects are being carried out in this area--one of them on brain and the other on muscle. The brain project on Metabolic Correlates of Brain Functional Activity is based on Sokoloff's demonstration that regional brain metabolism can provide a mirror for alterations in brain functional activity. The method used in these studies involves mapping of brain glucose consumption. As the brain under normal circumstances is dependent on the combustion of glucose for its energy, this method allows us to functionally map those brain regions which change their metabolic activity in association with specific physiological and/or experimental conditions.

Within the past year we have used 2-deoxy-D-glucose (2-DG) as a functional marker in the suprachiasmatic nucleus. A number of studies have suggested that there exists a central rhythm generator (an endogenous neural clock) for

generating and regulating circadian rhythms, and on the basis of lesion studies the suprachiasmatic nuclei (SCN) have been hypothesized as one possible locus for such a clock. In this project, in collaboration with Dr. H. Gainer, NICHD, a directly measurable property of the SCN, glucose consumption measured by the 2-DG autoradiographic method, was examined to determine if it would be affected by environmental light and exhibit circadian rhythmicity in intact (unlesioned) animals. It was found that the SCN glucose consumption could be "turned on" by exposure of the animal to light and that there was some evidence compatible with an endogenous rhythm of SCN glucose consumption even in the absence of environmental light. More experiments to prove the existence of an endogenous circadian rhythm of SCN glucose consumption and to determine what biochemical event(s) account(s) for the circadian rhythm of SCN glucose demand are still necessary.

The second LNP project on Neurochemical Correlates of Activity is concerned with Muscle Function and Metabolism. The aim of this project is to study controlling factors in the muscle excitation-contraction process and to understand the evolution of and recovery from muscle fatigue. Fatigue is produced in single muscle fibers by tetanic stimulation. We are interested in relating the duration of stimulation and tension output to the rates of consumption of available substrates like glycogen, ATP, and phosphocreatine. When a whole striated muscle is stimulated repetitively, its contractile force decreases and it is said to be fatigued. Fatigue does not occur in dystrophic muscles and is characteristic of fast but not slow mammalian and amphibian muscle fibers. The complexity of the excitation-contraction process in fast muscles makes it difficult to determine at what stage fatigue sets in. Single muscle fibers of the frog semitendinosus muscle, rather than whole muscles, were selected to study fatigue and recovery. Use of single fibers avoids rate-limited diffusion and the heterogeneous fiber population of whole muscle. In single muscle fibers we were able to distinguish the fatigue process following tetanic stimulation from the process of post-tetanic potentiation. Then we correlated, for the first time in fibers, tension with the instantaneous metabolic profile during various stages of fatigue and of recovery from fatigue. We showed that fatigue under the given experimental conditions was directly related to residual PCr concentration, but was not associated with significant depletion of glycogen or of ATP.

The relation between contraction, calcium release and uptake, and electrical activity of a single fiber, and kinetic changes in intracellular metabolic and calcium compartments is important not only for understanding the basic mechanisms of excitation-contraction coupling and fatigue and recovery of muscle, but also to see how metabolites are employed generally to support cellular activity. Our finding that fatigue develops despite high levels of ATP corresponds to observations of "power failures" in the stimulated peripheral nerve or sympathetic ganglion. In these tissues, function fails before energy supplies are exhausted. The protection of energy supplies at the expense of function may preserve the life of the neuronal or muscle cell.

III. Functional Neuroanatomy

Within the past decade there have been rapid advances in neuroanatomical methodology, which has in turn led to greater understanding of how neural

structure is related to neural function. Studies in "functional neuroanatomy" are concerned with elucidation of structure-function relationships in the nervous system.

The first major area of LNP work in functional neuroanatomy deals with Transport Mechanisms Across Membranes and the Blood-Brain Barrier. The blood-brain barrier at the cerebral vasculature, choroid plexus, and arachnoid membrane covering the brain regulates the ionic environment of the nervous system, has specific mechanisms for the delivery of amino acids and glucose to support brain mechanisms for the delivery of amino acids and glucose to support metabolism, and prevents access of blood-borne proteins to the brain, as well as access of some water-soluble drugs (e.g., penicillin and methotrexate).

The aim of this project is a) to understand the basic morphological and physiological properties underlying barrier function and protecting its integrity; b) to develop a method to open the barrier that may prove useful in pharmacology of the central nervous system; c) to understand how the barrier is affected by different stresses to the brain, including the loss of autoregulation of cerebral blood flow; and d) to understand the blood-ocular barrier and compare it with the blood-brain barrier.

The results of the project are significant because an understanding of how the barrier regulates the brain environment and controls brain metabolism is important to understanding function and homeostasis of the central nervous system. We have demonstrated osmotic barrier opening and have developed the first method that can reversibly allow the entry of normally excluded drugs into the central nervous system. This method should be useful as a technique for studying pharmacology of the central nervous system. Entry of antibodies could be used to study measles or other viral growth in the brain, and entry of enzymes could be used to study how the brain cells deal with exogenous enzymes for eventually understanding lysosomal storage diseases. Entry of norepinephrine has already been used to demonstrate central effects of that drug on cerebral blood flow.

We have also demonstrated that osmotic barrier opening is due to widening and increased permeability of cerebrovascular tight junctions, and suggested that tight junctions also are made more permeable by conditions which alter autoregulation of cerebral blood flow. This hypothesis has not yet been tested, but if it is correct the effects on cerebral function of clinical hypertension, convulsive activity, trauma, and other conditions which alter autoregulation should be reevaluated in terms of the influence of altered barrier function.

Finally, we have for the first time distinguished between the roles of the pigmented and nonpigmented epithelial layers of the ciliary body of the eye in regulating aqueous secretion. We have shown that the nonpigmented layer alone can control secretion and maintain normal intraocular pressure. Our findings that intraocular pressure can be reduced below normal over a period of months suggest that hypertonic perfusion of ocular tissue might be of eventual use in treating acute glaucoma.

A second project coming under the heading of Functional Neuroanatomy is Functional Studies of the Substantia Nigra. This project aims to understand both the normal function and the pathophysiology of the substantia nigra by

using the 2-DG tracer technique for measuring brain glucose consumption (see Project No. Z01 MH 01085-04 LNP). The substantia nigra is of research interest for a number of reasons. As part of the basal ganglia anatomically, its role in brain function remains quite unclear. The cell bodies of the substantia nigra contain dopamine, and their axons form a well-described dopaminergic nigrostriatal tract. Clinical interest in this system has been generated by the observation that decreased dopamine in the striatum and substantia nigra has been correlated with the presence of rigidity and akinesia in Parkinson's disease, and that basal ganglia degeneration may be an important factor in the pathogenesis of this disorder. Finally, a wide variety of brain functions has been attributed to dopamine, including regulation of feeding, reward mechanisms, and in the pathogenesis of schizophrenia.

To initially characterize the pattern of altered brain glucose consumption which might result from destruction of ascending dopaminergic pathways, large unilateral electrolytic lesions of the lateral hypothalamic area in the region of the ascending pathways were made. The brains of these animals revealed that the glucose consumption of a number of structures rostral and ipsilateral to the lesion was depressed compared with that of the contralateral side. The most significant effects were found in sulcal prefrontal cortex, frontal cortex, parietal cortex, and caudato-putamen; but not all forebrain structures were affected, and no structure caudal to the lesion was involved. The right-left metabolic asymmetry was present as early as 2 hours postoperatively and persisted virtually unchanged for at least 14 days. Finally, study of one other biochemical pathway, the incorporation of intravenously injected [^{14}]L-leucine into its formalin-fixed metabolic derivatives (mostly proteins), in the lesioned animals revealed that there was no lesion-induced left-right disruption of this pathway. Since at least this metabolic pathway appeared preserved in lesioned animals, the observed lesion-induced disruption of forebrain glucose consumption was probably not a consequence of inert ipsilateral brain tissue. Furthermore, the disruption of the metabolic pathways for forebrain glucose consumption may have been a specific effect of the lesions and not merely a nonspecific reflection of a lesion-induced alteration of whole brain mechanism.

To determine what role ascending dopaminergic pathways might play in the development of the metabolic sequelae of the lateral hypothalamic lesions, 6-hydroxydopamine was unilaterally injected into the substantia nigra. The asymmetry of glucose consumption could be partially reproduced in some of the forebrain structures, most significantly in the caudato-putamen, by such lesions. Destruction of the cell bodies which give rise to the ascending dopaminergic nigrostriatal bundle is one consequence of 6-hydroxydopamine lesions of the substantia nigra, so it is possible that the destruction of this bundle by the lateral hypothalamic lesions was partially responsible for the disrupted pattern of caudato-putamen glucose consumption observed after lateral hypothalamic lesions.

As one test of this hypothesis, apomorphine, a dopamine receptor agonist, was administered to animals with lateral hypothalamic lesions to determine whether pharmacological stimulation of denervated striatal dopaminergic receptors could correct the metabolic deficit in the caudato-putamen. When apomorphine was given to the lesioned animals 14 days postoperatively, the glucose consumption in sulcal prefrontal cortex, frontal cortex, and parietal cortex remained

ipsilaterally depressed, but the right-left asymmetry of the glucose consumption in caudato-putamen had disappeared (and had even reversed in anterior caudato-putamen). Interestingly, when apomorphine was given to the lesioned animals 3 days postoperatively, there was no effect on the metabolic asymmetry of any of the forebrain structures, including that of the caudato-putamen.

Therefore, the observed asymmetry of glucose consumption in caudato-putamen after unilateral electrolytic lesions of the lateral hypothalamus can be 1) partially reproduced by 6-hydroxydopamine injections into the substantia nigra, and 2) reversed by intravenous apomorphine. The observation that a 1 mg/kg dose of apomorphine restores caudato-putamen glucose consumption 14 days after lesions had been made but is ineffective 3 days postoperatively may possibly be explained by a leftward shift in apomorphine's dose-response curve as a result of the gradual onset of "denervation supersensitivity" of the postsynaptic dopaminergic receptors in the caudato-putamen.

A new project under the broad heading of Functional Neuroanatomy was begun in January of this year. The purpose of this project is to allow utilization of the newest neuroanatomical methods in conjunction with studies of brain physiology based on traditional microelectrode recording techniques. The anatomical methods utilized in this project include amino acid autoradiography and horseradish peroxidase (HRP) tracing of neuroanatomical connections and radioactive 2-DG for functional mapping of brain sites which exhibit changes in metabolic activity in relation to different behaviors. One of the long-range goals of this project is to discover still additional "markers" to identify the individual and/or neuronal networks mediating specific forms of behavior. Our traditional microelectrode recording techniques have been fruitful, but suffer from the limitation that only one or at most a few cells can be studied at any one time. In the future it seems possible that anatomical methods will enable us to "mark" active cells with specific chemicals taken up according to their activity and then to identify the marked cells in histological sections. Such techniques would allow us to look at millions of cells in a particular animal preparation and to obtain a much fuller understanding of the patterns of cellular activity involved in mediating both simple and complex behavior.

Laboratory of Clinical Psychopharmacology
Richard Jed Wyatt, M.D., Chief

The year 1977 has been a successful year for the Laboratory of Clinical Psychopharmacology. We have been blessed with a superb group of young physicians who provided the highest quality of care for our patients, have been interested in the problem of schizophrenia, and have sought innovative answers as to how we might better treat them. Our major scientific success has been the splitting of severe chronic schizophrenic groups using platelet monoamine oxidase as a discriminator. Platelet monoamine oxidase discriminates acute from chronic schizophrenics but within the severe chronic schizophrenics discriminate the paranoids from the nonparanoids. This is a finding that has been replicated not only in our lab but at least in one other laboratory. The meaning of this finding is still under investigation and with the full operation of our biochemistry unit operated by Drs. Karoum and Neckers, as well as numerous collaborations outside the laboratory, we hope to have a better understanding of the significance of this finding. We have continued numerous drug trials in the hope that we may be able to alleviate some of the schizophrenic symptoms. The most promising avenue has been the use of alpha-methyl-para-tyrosine in conjunction with conventional neuroleptic drugs. In a small group of schizophrenics, alpha-methyl-para-tyrosine appears to have an additive effect to the neuroleptics on some measures of behavior. Unfortunately, this is unlikely to provide a practical form of treatment because of the toxicity of alpha-methyl-para-tyrosine. If, however, further trials with alpha-methyl-para-tyrosine indicate that it is, in fact, a useful treatment, similar drugs with less toxicity may be developed and found to be useful.

Dr. Stoff has continued development of animal models of schizophrenia, particularly involving phenylethylamine (PEA) and dimethyltryptamine (DMT), in order to examine clinical criteria relevant to psychiatric illness.

Our studies of sleep--the project report which will be found in the Adult Psychiatry Branch's Annual Report, have also had considerable success. Drs. Gillin and Mendelson have further explored the relationship of acetylcholine to human sleep as well as the relationship of biogenic amines to the secretions of neurohormones produced in the hypothalamus and secreted by the pituitary gland.

Dr. Stillman has continued his inventive studies in an attempt to understand why people use drugs of abuse and his finding that there is a suppression of the left hemispherical function with marijuana and that this suppression is different than that produced by alcohol use appear to be very promising.

Our aging studies still remain very much in their incipient stages due to the lack of personnel with which to carry them out. However, it is hoped that in the next year, even without increased personnel, we will be able to begin these in a small way.

Laboratory of Neuropharmacology
Richard Jed Wyatt, M.D., Acting Chief

This summary is the ninth in the history of the laboratory. Since the last report by Dr. George Siggins, he has joined Dr. Floyd Bloom at the Salk Institute in LaJolla. Dr. Barry Hoffer subsequently became acting chief until the late summer when he left for the University of Colorado Medical Center. I have been serving in the capacity of acting chief since that time. The activities of the lab have been maintained primarily by Drs David Taylor, who has now left to join Dr. Hoffer in Colorado, Dr. Margret Schlumpf who left during the winter, Mr. Paul Oliver, Ms. Eleanor Krauthamer, and the Section on Synaptic Pharmacology headed by Dr. Forrest F. Weight. Dr. Schlumpf and Dr. Taylor continued to work on projects that they had been assigned by senior investigators before they left. Dr. Schlumpf's work is as described in last year's report. Dr. Taylor has submitted project reports for this year. His primary efforts were in studying the physiology and morphology of transplants from fetal transplants into the anterior chamber of a rat. He will continue this work when he arrives in Colorado. Ms. Krauthamer has been primarily assigned to work in Dr. Costa's laboratory during the next year. Mr. Oliver has used a guinea pig hippocampal slice to study the production seizures. He has successfully demonstrated that the seizures can be contridied by conventional anticonvulsive drugs. He will continue this work during the next year. Dr. Weight's activities are described fully below. As far as the organization of the laboratory goes during the next year, we will continue our efforts to identify an area and direction so that we may choose a new laboratory chief. Dr. Eberhart has set up a committee which has interviewed a number of investigators primarily in the areas of anatomy and pharmacology so that we might identify a direction. Once this direction is better identified a formal search committee will be set up which will solicit applications for the new chief. It is expected, however, that this process will take considerable time.

SECTION ON SYNAPTIC PHARMACOLOGY
Forrest F. Weight, M.D., Chief

This summary report will outline the progress of the Section toward the accomplishment of its goals. The mission of the Section on Synaptic Pharmacology is the elucidation of the cellular basis of nervous system function. This goal has been approached by multidisciplinary studies including physiology, pharmacology, anatomy and biochemistry focusing particularly on understanding the communication between nerve cells at synapses. The purpose of these investigations is to provide fundamental knowledge on the cellular basis of nervous system function, which will provide a foundation for our future understanding of the biological basis of mental illness.

The investigations of the Section on Synaptic Pharmacology have centered around five major questions, which will serve as the organizational basis for this summary: (1) the control and modulation of transmitter release; (2) the membrane permeability changes resulting from transmitter action; (3) the molecular mechanisms involved in the synaptic control of membrane permeability; (4) long-lasting control of postsynaptic membrane permeability and the modulation of synaptic transmission efficacy; and, (5) the effect of psychotherapeutic agents on synaptic transmission mechanisms.

I. HOW IS TRANSMITTER RELEASE CONTROLLED AND MODULATED?

One of the important aspects of synaptic transmission is the release of the chemical transmitter. In order to understand the mechanisms involved in the control and modulation of transmitter release, we have studied three aspects of transmitter release: (1) the role of potassium in the control of transmitter release; (2) the role of temperature in transmitter release; and (3) physiological mechanisms modulating the release of synaptic transmitter. These studies will be briefly summarized.

Role of Potassium in the Control of Transmitter Release.

Potassium ions have been found in previous studies to accumulate extracellularly in the nervous system with repetitive nervous activity. There is little information, however, on the physiological effects and functional significance of increased extracellular potassium. In this investigation, we studied the effect of changing extracellular potassium concentration on synaptic transmitter release at the squid giant synapse. We found that an increase in extracellular potassium decreases the release of transmitter. This depression of transmitter release is due to a change in the amplitude of the presynaptic

action potential. Such a depression of transmitter release could be important functionally when nervous activity results in an accumulation of extracellular potassium.

Role of Temperature in Transmitter Release.

Transmitter release has been found to be temperature sensitive in previous investigations; however, in those studies it was not possible to determine if the changes in transmitter release were due to effects of temperature on the amplitude of the presynaptic action potential, because at most synaptic junctions the presynaptic spike can not be recorded. We studied this problem at the squid giant synapse, where it is possible to record the presynaptic action potential intracellularly. We found that a change in presynaptic spike height does not appear to be the major factor involved in the sensitivity of transmitter release to temperature. The effect of temperature on transmitter release is therefore likely to involve other factors such as temperature sensitivity of the release mechanism.

Physiological Mechanisms Modulating the Release of Synaptic Transmitter.

The modulation of transmitter release by physiological activity may play an important role in the processing of information in the nervous system. The knowledge that repetitive activity of a neuron or axon results in an accumulation of extracellular potassium, together with the evidence that increasing extracellular potassium concentration decreases transmitter release (see above), prompted us to investigate whether the accumulation of extracellular potassium resulting from a train of impulses in a postsynaptic structure might modulate the release of transmitter from the presynaptic terminal. We found that repetitive action potentials in the postsynaptic axon of the squid synapse results in a depression of transmitter release from the presynaptic terminal, and this effect results from the accumulation of extracellular potassium ions. The modulation of transmitter release by repetitive postsynaptic action potentials can function as a negative feedback mechanism to inhibit impulse transmission through a synapse. In this way, the accumulation of extracellular potassium resulting from physiological activity may modulate synaptic transmission and function as an integrative mechanism in the nervous system.

II. WHAT ARE THE MEMBRANE PERMEABILITY CHANGES RESULTING FROM TRANSMITTER ACTION?

A second important aspect of synaptic transmission is the effect of the synaptic transmitter on the postsynaptic neuron to generate postsynaptic responses. In order to be able to study in detail the mechanisms by which synaptic transmitter action on postsynaptic receptors leads to the generation of postsynaptic responses, it is necessary to be able to stimulate selectively a specific synaptic pathway, to know the identity of the synaptic transmitter, and to know the types of postsynaptic receptors activated by the transmitter. To achieve this goal, we have investigated postsynaptic mechanisms of transmitter action, in a model where these factors can be precisely defined and studied, the sympathetic ganglion of the bullfrog. In these investigations, we have identified and characterized the synaptic pathways, the synaptic transmitters, and the postsynaptic receptors involved in the generation of postsynaptic responses. We have then used these defined synaptic pathways to study and elucidate the membrane and molecular mechanisms involved in different types of postsynaptic responses, and to study the synaptic mechanisms of psychotherapeutic drug action.

Identification and Characterization of Synaptic Pathways, Synaptic Transmitters and Postsynaptic Receptors.

A combination of electron microscopic, histochemical, pharmacological, and electrophysiological methods were utilized in this investigation. The neurons in the sympathetic ganglia of the bullfrog were found to be unipolar, and were innervated by a single preganglionic axon. The cholinergic preganglionic fiber stained for acetylcholinesterase and was observed in both the light and the electron microscope to spiral around the axon hillock of the ganglion cell before arborizing and making synaptic contact with the neuron. Most synapses were located on the soma, a situation well suited for studying the permeability mechanisms involved in the generation of postsynaptic responses.

With intracellular recording, two types of neurons have been identified, B cells and C cells; each type of neuron receives a single monosynaptic excitation from different presynaptic nerves. The presynaptic fibers in the sympathetic chain synapse with B cells, and the presynaptic fibers in the eighth nerve synapse with C cells. Selective stimulation of each of these presynaptic nerves elicits a fast excitatory postsynaptic potential (fast EPSP) in each cell type. Administration of acetylcholine (ACh) by iontophoresis mimics the fast EPSP, and both the fast EPSP and the ACh response are

blocked by nicotinic antagonists, indicating that ACh is the transmitter activating nicotinic postsynaptic receptors. In the presence of nicotinic antagonists, repetitive stimulation of the separate presynaptic nerves produces slow synaptic potentials of opposite types in the two cell types. Stimulation of the sympathetic chain produces a slow excitatory postsynaptic potential (slow EPSP) in B cells, and stimulation of the eighth nerve produces a slow inhibitory postsynaptic potential (slow IPSP) in C cells. Both the slow EPSP and the slow IPSP are mimicked by the iontophoretic administration of ACh to B and C cells, respectively. The muscarinic antagonist, atropine, blocks the effects of iontophoretically applied ACh and both the slow EPSP and the slow IPSP, indicating that both the slow EPSP and the slow IPSP are mediated by ACh activating muscarinic postsynaptic receptors.

The fluorescence histochemical method demonstrates that there are small intensely fluorescent (SIF) cells distributed randomly in the ganglia and located in the vicinity of blood vessels. Histochemistry with the chromaffin reaction reveals that the SIF cells are a type of chromaffin cell. Electron microscopy shows that ultrastructurally the SIF-chromaffin cells appear to be identical to the chromaffin cells in the adrenal gland. The SIF-chromaffin cells do not have the morphological characteristics of interneurons and thus are apparently not involved in the mediation of synaptic potentials. This is in accord with the anatomical and physiological data cited previously, that the fast and slow synaptic potentials are due to cholinergic innervation of the neurons.

Synaptic Control of Postsynaptic Membrane Permeability.

Synaptic excitation results from an increased permeability of the postsynaptic membrane at most chemically transmitting synapses that have been investigated. This has also been found to be the case for the fast EPSP in these sympathetic neurons, where the transmitter ACh, acting on nicotinic receptors, increases the postsynaptic permeability to sodium and potassium ions. Investigation of the slow EPSP has revealed that, in contrast to the increased permeability associated with most other EPSPs, the slow EPSP is generated by a decrease in membrane permeability. Electrophysiological analysis indicates that the mechanism of generation of the slow EPSP is a decrease in the permeability of the membrane to potassium ions. Because of the in vitro nature of the preparation, and the fact that the transmitter is known to be ACh, it has been possible to apply

ACh iontophoretically and change extracellular potassium ions to obtain additional data supporting this mechanism. Investigation of the slow IPSP indicates that it is also generated by a decrease in membrane permeability, in contrast to the increased permeability associated with most other IPSPs. Electrophysiological analysis of the slow IPSP indicates that the decreased permeability is predominantly to sodium ions. The iontophoretic administration of ACh and changing extracellular sodium has provided supporting data for this mechanism. There are a number of suggestions that similar synaptic mechanisms are involved in slow postsynaptic potentials in the central nervous system; however, because of various technical and experimental limitations, it has not been possible to adequately characterize those mechanisms. Now that various types of synaptic mechanisms have been elucidated in this preparation, it is possible to investigate the molecular mechanisms involved in the generation of these synaptic potentials.

III. WHAT ARE THE MOLECULAR MECHANISMS INVOLVED IN THE SYNAPTIC CONTROL OF MEMBRANE PERMEABILITY?

Role of Cyclic Nucleotides in Synaptic Potential Generation

In an attempt to determine the molecular mechanisms underlying synaptic potential generation, the possibility that cyclic nucleotides may act as intracellular second messengers has been investigated. In collaborative experiments with Dr. P. Greengard (Yale Univ.), it has been found that brief stimulation of preganglionic nerve fibers results in a rapid and significant increase in both cyclic guanosine monophosphate (cyclic GMP) and cyclic adenosine monophosphate (cyclic AMP). The increase in cyclic GMP and cyclic AMP content that occurs with stimulation in normal Ringer's solution is not observed in ganglia stimulated in a high magnesium, low-calcium Ringer. Atropine, a muscarinic cholinergic antagonist that blocks both the slow IPSP and the slow EPSP in this tissue, also blocks the increase in cyclic GMP, but not the increase in cyclic AMP. These data are consistent with the possibility that cyclic GMP may be involved in the postsynaptic action of acetylcholine at muscarinic cholinergic synapses. Further investigation is necessary to determine the postsynaptic receptors involved in the cyclic AMP activation.

In view of the preceding data, the possibility that cyclic nucleotides might mediate the permeability changes underlying the generation of the slow postsynaptic potentials was investigated by electrophysiological techniques. Consistent with this possibility, we found a potentiation of the slow IPSP

by theophylline, an inhibitor of phosphodiesterase - the enzyme that breaks-down cyclic nucleotides. However, theophylline also had direct membrane actions that might account for this potentiation. Furthermore, the administration of cyclic GMP, cyclic AMP, and their derivatives, had no consistent effect on membrane potential. Thus, it is, at present, uncertain as to whether the increases in cyclic nucleotides elicited by stimulation of presynaptic afferents are involved in the mediation of postsynaptic potential generation, or have other functional roles in these neurons.

Role of Electrogenic Sodium Pump in Slow Synaptic Inhibition.

Another molecular mechanism that might be involved in postsynaptic potential generation was also investigated. We studied the possibility that the slow IPSP might be due to the synaptic activation of the electrogenic sodium pump. In this project, the activation and inhibition of the electrogenic sodium pump was first studied and characterized. We found that potassium-free Ringer and ouabain are effective inhibitors of the electrogenic sodium pump in this tissue. These inhibitors were then tested on the generation of the slow IPSP. It was found that they do not block the slow IPSP. These data indicate that the slow IPSP does not result from a synaptic activation of the electrogenic sodium pump.

IV. DOES LONG-LASTING CONTROL OF POSTSYNAPTIC MEMBRANE PERMEABILITY MODULATE THE EFFICACY OF TRANSMISSION AT SYNAPSES?

The synapse functions as the communication link between nerve cells. An understanding of the mechanisms by which synaptic transmission can be modulated may therefore be important in the elucidation of the cellular mechanisms underlying complex nervous system functions. The vast majority of postsynaptic responses are of very short duration (msec) and thus postsynaptic interactions are brief and require a high degree of synchrony. Furthermore, at most synapses the postsynaptic potentials are generated by increases in membrane permeability and when such postsynaptic potentials combine the result is at most their sum, and often it is less. As indicated previously, the slow postsynaptic potentials are generated by decreases in membrane permeability, which is measured by electrophysiological techniques as an increase in the membrane's electrical resistance. Such an increase in membrane resistance would be expected to increase the amplitude of fast EPSPs. We tested this possibility and found that the slow decreases in

membrane permeability can increase the effectiveness of impulse transmission at synapses. Slow decreases of membrane permeability of sufficient magnitude increased the amplitude of monosynaptic fast EPSPs. By this postsynaptic mechanism, activation of one synaptic pathway causes an increase in the transmission of impulses, lasting several minutes, across another synapse. By augmenting rather than diminishing, a convergent synaptic input, a decrease in membrane permeability can make that synaptic input more effective in the generation of postsynaptic action potentials. Increasing transmission in a synaptic pathway by decreasing postsynaptic membrane permeability may be an important mechanism in information processing in the nervous system. Long-term control of such postsynaptic membrane properties might provide a cellular mechanism for the storage of information.

V. WHAT ARE THE SYNAPTIC MECHANISMS OF PSYCHOTHERAPEUTIC DRUG ACTION?

The cellular mechanisms of action of lithium in the nervous system are of interest because of its importance in treating manic-depressive psychosis. Replacing sodium in the Ringer's solution by lithium, we found first that lithium produces a hyperpolarization and a decrease in the ionic conductance of the membrane, indicating that lithium ions are less permanent than sodium ions. Secondly, we found that lithium concentration builds up intracellularly, presumably because it is not extruded by the electrogenic sodium pump. Third, lithium inhibits the generation of the slow IPSP. The mechanism of this lithium action has not yet been elucidated and is currently the subject of active investigation.

CONCLUSIONS

The preceding summary indicates that fundamental neurobiological investigations, using a multidisciplinary approach, have defined synaptic pathways, identified synaptic transmitters and characterized postsynaptic receptors. This basic information has been used to investigate the different types of membrane and molecular mechanisms by which synapses transfer information from one nerve cell to another. The characterization of several types of synaptically activated membrane permeability changes has led to the elucidation of a neuronal mechanism that may function as a cellular mechanism for information storage in the nervous system. Additionally, the understanding of the cellular mechanisms of synaptic transmission provides a locus for investigating the synaptic basis of psychotherapeutic drug action in the nervous system.

LABORATORY OF PRECLINICAL PHARMACOLOGY

Chief: E. Costa M.D.

1976-1977

I am pleased to report that this year the staff of the Laboratory of Preclinical Pharmacology IRMH not only successfully pursued the research trends described in last year's report but, in addition, has expanded into new areas of neurochemical pharmacology and has made some original and significant contributions to these new areas.

With the help of Dr. J. Schwartz, adequate facilities for tissue cultures of neuroblastoma and glioma cell lines were developed. Using primary cultures of beef chromaffin cells developed by Dr. Waymire (University of California at Irvine), Drs. Guidotti and Chuang have begun a systematic investigation of how the nuclear translocation of catalytic subunits of cAMP dependent cytosol protein kinase participates in the transsynaptic regulation of gene expression. In another series of tissue culture experiments, Dr. Schwartz found that glioma C6 and neuroblastoma cells contain amounts of nerve growth factor which can be measured by radioimmunoassay and by bioassay using organ cultures of chick embryo sensory ganglia. These glioma cells possess adenylate cyclase which is functionally linked with adrenergic beta-receptors and not with prostaglandin receptors; in contrast, the adenylate cyclase of neuroblastoma cells is activated by prostaglandins but not by beta-adrenergic receptor agonists. In each of the two cell types, the increment in the cAMP content that follows adenylate cyclase activation causes within 3 hrs an increase in the content of the protein that is precipitated by the immunoglobulin directed toward nerve growth factor. Mr. P. Della Bella (student on leave from the Univ. of Milan, Italy) and Dr. Schwartz have shown that this increase in nerve growth factor content is not due to new protein synthesis. Moreover, preliminary evidence suggests that the peptidase activity associated with a portion of the nerve growth factor molecule (gamma chain) is activated as a result of the cAMP increase. We are investigating whether more nerve growth factor is produced from a prohormone stored in glioma C6 cells. An operant model to interpret the physiological implications of this finding is: following excessive nerve activity, the excess transmitter released into a synaptic gap can reach specific receptors in the outer membrane of surmounting glial cells. The increase in second messenger triggered by the activation of specific receptors linked with adenylate cyclase, activates the peptidase which, in turn, catalyzes the formation of more nerve growth factor. Secretion of this factor from glial cells into the medium follows. The nerve growth factor that reaches the extracellular space can be taken up by the hyperactive axon terminals and be antidromically transported to the cell body of the hyperactive neuron. Here the nerve growth factor assures the production of those proteins that are used at rates faster than normal when the nerve activity becomes excessive.

During this fiscal year, our program was enriched by a number of contributions concerning new putative neurotransmitters with polypeptide structure. These novel research trends were carried out by Drs. Hong and Fratta under the guidance of Dr. Yang. After obtaining specific antibodies directed

toward leu and met-enkephalins, substance P and neurotensin, they identified in the CNS specific neuronal pathways which store and release these putative neurotransmitters. This extra dimension added to the potential of our program has given us the capability of studying the neurochemical pharmacology of peptidergic transmission. The extensive distribution of these peptidergic neurons throughout the brain and spinal cord seems to rival and, perhaps, to exceed that of the better known catecholaminergic neurons. A significant advance has been the demonstration that cell bodies for enkephalinergic neurons innervating the striatum reside in this structure. Conversely, striatal neurons that store substance P innervate substantia nigra. Other substance P terminals innervate the nucleus interpeduncularis and originate from cell bodies located in the habenula.

In our program, the concept of peptidergic neurotransmission has reached fruition as an area of multidisciplinary research since Dr. Hong found that in rats chronically injected with certain antischizophrenic compounds, the striatal content of met-enkephalin increases and that of substance P in substantia nigra decreases. The emerging pattern indicates that both changes occur only after chronic treatment with those antischizophrenic compounds that after chronic treatment also elicit supersensitivity of striatal dopamine receptors in rats and tardive dyskinesia in man.

Drs. Gale, Hong and Guidotti have shown that probably a stimulation of substance P receptors in nigra is involved in decreasing the Km of striatal tyrosine hydroxylase following haloperidol. A functional role of dopaminergic regulation through polysynaptic loops rather than through autoreceptors as emphasized in other laboratories, was elucidated by lesion studies performed by Drs. Gale, Chuang and Guidotti. These experiments have indicated that DA autoreceptors are either nonexistent or, if they do exist, they have a secondary role in regulating dopaminergic function.

Another new development was the study at the molecular level of the mode of action of cholera toxin. Dr. Chuang and Ms Seaborg (Student from American University, Washington, D.C.) have revealed that the "A" chain of the toxin hydrolyses nicotine-amide adenosine diphosphate; thereby the toxin becomes covalently bonded to diphosphate ribose.

Important advances also occurred in other research programs initiated in previous years: particularly the study of the mode of action of antidepressants (Drs. Robinson and Cheney), antischizophrenics (Drs. Mao, Revuelta, and Marco), anxiolytics (Drs. Mao, Revuelta and Marco), opiates (Dr. Moroni and Cheney) and cannabinoids (Drs. Revuelta, Moroni and Cheney), GABA (gamma-aminobutyric acid) and acetylcholine (ACh) in brain nuclei. Drs. Mao and Peralta (a Guest Worker from Spain) have shown that stimulation of neostriatum increases the turnover rate of GABA in substantia nigra selectively. Furthermore, Drs. Cheney and Moroni have shown that intraseptal injection of drugs can be used to study the trans-synaptic regulation of the septal hippocampal cholinergic system. In addition, Dr. Moroni has found that stimulation of the septum increases the ACh turnover of hippocampus but decreases that of striatum. Drs. Malthe-Sorensen (a Guest Worker from

Norway), Wood (guest worker from Canada) and Cheney have successfully obtained a preliminary brain map of the functional links between various peptidergic and cholinergic neurons in a number of brain structures. Finally, the high specificity of mass fragmentography has allowed Drs. Commissiong, Galli and Neff to identify a dopaminergic pathway in rat spinal cord and Drs. Hellstrom and Hanbauer (NIHL, Bethesda, Md.) to study how hypoxia releases DA stored in glomus cells of carotid body.

Another methodological advance of great significance was the development of high pressure liquid chromatography by Drs. Bohan and Meek as a method to study peptidergic transmission. When this assay is combined with radioimmunoassay, its specificity and sensitivity is unparalleled by any other existing method. The study of brain serotonin turnover rate in brain nuclei (Drs. Marco and Meek) is revealing interesting aspects of serotonergic function which indicate possible important deviations in the serotonergic neurons of the cortex and septum from the classical model which was formulated when the turnover of serotonin was measured in the whole brain.

SECTION ON MOLECULAR NEUROBIOLOGY (ACTING CHIEF: E. COSTA, M.D.)

Nuclear Translocation of Protein Kinase

Previous work by Dr. Guidotti and his collaborators had shown that the increase in the synthesis rate of medullary tyrosine hydroxylase elicited transsynaptically was mediated by an increase in phosphorylation of acidic nuclear protein caused by the translocation of the catalytic subunits of cAMP-dependent type I protein kinase (PK). Drs. Chuang and Hollenbeck have studied in detail the protein phosphorylation in purified nuclei of chromaffin cells purified from beef adrenal. They found that these nuclei contain three types of PK. All three enzymes are cAMP independent but they can be differentiated with regard to their sedimentation coefficient, elution characteristics from DEAE cellulose column and substrate specificity. When the three enzymes were tested using various histone mixtures and casein as a $P_{0.4}^{-3}$ acceptor two enzymes preferentially catalyzed casein phosphorylation the other one preferred histone. None of the three enzymes were inhibited by the specific inhibitor of cAMP dependent PK or by the addition of regulatory subunits of cAMP dependent PK. When medullary cell chromatin was transcribed with homologous RNA polymerase II, the phosphorylation of chromatin protein curtailed the endogenous template restriction. Since chromatin phosphorylation appeared to regulate transcription, Drs. Chuang and Hollenbeck decided to build a model to study whether and how catalytic subunits of cAMP dependent PK were transferred into a medullary nuclei incubated with homologous PK. It was found that the catalytic subunits of the enzyme were transferred to the nuclei where they were tightly bound to the chromatin. The newly taken up enzyme did not require cAMP and could be inhibited by recombination with regulatory subunits of PK. After enzyme recombination experiments, the activity of the newly taken up enzyme could be expressed only in the presence of $7 \mu M$ cAMP. Since the endogenous protein which specifically inhibits cAMP dependent PK failed to inhibit the activity of the newly taken up enzyme, Drs. Chuang and Hollenbeck have inferred that the site in which catalytic subunits of PK bind with chromatin may be important for the PK inhibition by the

endogenous inhibitor. The model they have proposed suggests that transmitters by causing a long term activation of adenylate cyclase can cause a long lasting dissociation of cAMP dependent PK in cytosol; this dissociation is an essential precondition for nuclear translocation of cytosol PK. The free catalytic subunits thus produced can be taken up by nuclei where by an unknown mechanism they localize in specific nuclear sites where by controlling phosphorylation of protein closely associated with chromatin, change the rate of RNA transcription.

Carotid Body Regulation

Drs. Hellstrom and Hanbauer have shown that the decrease in dopamine content that occurs in carotid body of rats kept in hypoxic conditions is due to a release of the amine from their storage in glomus cells. Moreover, they have shown that the DA release is a specific response of glomus cells to hypoxia because it occurs also in the denervated carotid body (ganglionectomy and carotid nerve denervation).

Molecular Mechanisms of Dopamine Receptor Supersensitivity

Elicited by Antischizophrenic Drugs

²Drs. Uzunov and Gnegy have shown that an endogenous protein which binds Ca^{2+} and activates cyclic nucleotide phosphodiesterase or facilitates adenylate cyclase stimulation by DA is stored in postsynaptic membranes. This protein can be released from this binding site by membrane phosphorylation with cAMP dependent PK. Neither the protein activator nor the adenylate cyclase stored in the membrane act as PO_4^{3-} acceptors. When the activator protein is released from synaptic membranes of rat striatum by phosphorylation, the adenylate cyclase can no longer be stimulated by DA. Thus, Drs. Uzunov and Gnegy have inferred that the Ca^{2+} binding activator functions in the transsynaptic regulation of the second messenger response by regulating the adenylate cyclase associated with postsynaptic DA receptors and by lowering the K_m of the phosphodiesterase. This inference was upheld by experiments performed with chronic treatment with DA receptor blockers endowed with antischizophrenic action. The antischizophrenic compounds can be classified as cataleptogenic and noncataleptogenic. In animals, the cataleptogenic antischizophrenics cause a high incidence of extrapyramidal side effects and on prolonged treatment elicit DA receptor supersensitivity; in man they also cause extrapyramidal side effects which are short lasting but with chronic treatment often cause tardive dyskinesia. Haloperidol and d-butaclamol are cataleptogenic and cause tardive dyskinesia; clozapine is a noncataleptogenic antischizophrenic and fails to cause DA supersensitivity and tardive dyskinesia; l-butaclamol is an inactive enantiomer. Other authors have previously reported that those antischizophrenic compounds that elicit in man a high incidence of extrapyramidal side effects also cause tardive dyskinesia and in rats cause a supersensitivity to injected apomorphine. Drs. Gnegy and Gallorini (Guest Worker from the University of Pavia, Italy) could show that this supersensitivity to the behavioral action of apomorphine is associated to a supersensitivity of adenylate cyclase to DA stimulation and to an increase in the striatal membrane content of the Ca^{2+} dependent endogenous activator. These experiments have revealed for the first time a biochemical correlate of postsynaptic

receptor supersensitivity in animals. It is of great interest to study whether an increase in striatal content of Ca^{2+} binding activator is a biochemical marker for tardive dyskinesia in man. Moreover, it is important to note that clozapine, a dopamine receptor blocker which may be noncataleptogenic because of antimuscarinic properties can prevent the Ca^{2+} supersensitivity and the accumulation in postsynaptic membranes of the Ca^{2+} binding protein activator. This finding invites speculation as to the role of muscarinic receptors in striatum: perhaps, their activation causes an increase in the activity of cells innervated by DA terminals, when this activity is curtailed by blockade of muscarinic receptors, also the supersensitivity to DA fails to occur.

Brain Angiotensin Converting Enzyme and Neuropathology

Dr. Yang and Mrs. Majane investigated the brain content of angiotensin converting enzyme in brain parts. This enzyme is present in substantia nigra and caudate. the highest concentration is in the cerebellum. Probably, in substantia nigra the neurons that contain converting enzymes are in part intrinsic to this tissue, in part, project to the caudate. This enzyme and the Substance P content of nigra isolated from Huntington Chorea brain was studied and found to be very low. Hence, in agreement with other reports, Huntington chorea cannot be viewed as an idiopathic deficiency of the GABAergic system.

Antischizophrenic Drugs and Peptidergic Transmission

In the striatum, drug induced supersensitivity of postsynaptic DA receptors appears also to be associated with changes in intrinsic peptidergic transmission. Drs. Hong and Yang have shown that after chronic haloperidol there is a decrease in substance P content of substantia nigra and a marked increase in met-enkephalin content of striatum. These authors had previously shown that among the efferent projections from nucleus caudatus to substantia nigra there is an important peptidergic pathway which releases substance P. Hence, the finding that Substance P content of nigra but not that of other brain structures is decreased after chronic haloperidol but not after chronic clozapine was interpreted to indicate a direct participation of Substance P neurons in the action of cataleptogenic antipsychotics. Drs. Hong and Yang have also shown that practically all of the met-enkephalin stored in striatum is present in intrinsic striatal neurons. Chronic haloperidol increases enkephalin in striatum but not in hypothalamus. Thus, this increase in met-enkephalin may be a reflection of transsynaptic activation of intrinsic enkephalinergic neurons. To confirm this suspicion they are presently investigating whether specific striatal peptidases are inhibited or activated following chronic haloperidol. In fact, Drs. Yang, Hong and Meek had shown that the first step in the degradation of met-enkephalin is the cleavage of tyrosine presumably by an action of an aminopeptidase. Moreover, it is possible that met-enkephalin is synthesized via a cleavage of the pentapeptide from a prohormone which could be a fragment of beta-lipotropin.

Morphine Dependence and Regulation of Metenkephalin

Reports from other laboratories had shown that foot shock or morphine dependence increased metenkephalin content of brain suggesting that this endogenous opioid during narcotic dependence may be regulated through a change in the steady state. When these experiments were repeated by Drs. Fratta, Hong and Yang, the metenkephalin content failed to increase. This was not surprising because the work done in other laboratories was performed without instantaneous inactivation of brain enzymes by microwaves and with a low yield extraction procedure. In fact, the metenkephalin content reported by Drs. Hong, Yang and Fratta is about 100-fold higher than that previously reported by other workers who did not use microwaves. The highest content of metenkephalin was found in globus pallidus (70 pmoles/mg protein) followed by caudate, N. accumbens and central gray; the lowest content of metenkephalin was in telencephalic white matter, hippocampus and medial thalamic nuclei.

Regulation of Neuronal Trophism By Glial Cells: Activation of Nerve Growth Production by Stimulation of Neurotransmitter Receptors in Glial Cells

The existence of neuronal glial interactions is an axiom of neurochemistry. However, the trophic aspects of such interactions have never been characterized with any detail. Reports from other laboratories have indicated that glial cells contain receptor mechanisms for various transmitters, including beta-adrenergic receptors. Dr. Schwartz showed that a culture of glial cells (glioma C6) which possesses receptors for catecholamines also contains measurable amounts of nerve growth factor. This protein increases in the cells and in the growth media following stimulation of the beta receptors. Dr. Schwartz showed that this increase is not associated with an increase in total protein content and depends on an increase in the cAMP content. Since the increase in nerve growth factor does not require intact RNA transcription and translation and does not include newly synthesized polypeptides, we have thought that also nerve growth factor is stored as a prehormone and converted into the active molecule enzymatically. Existing evidence appears to indicate that the gamma chain of the nerve growth factor molecule is endowed with aminopeptidase activity. Dr. Schwartz has seen that this activity may be enhanced when the cellular content of cAMP is enhanced, she is presently investigating whether phosphorylation mediates this peptidase activation.

GROUP ON HIGH PRESSURE LIQUID CHROMATOGRAPHY (Head: J. Meek, Ph.D.)

The goal of this group is to develop and use simple specific and sensitive methods to study transmitter function. This year the activity of this group was focussed toward the study of the action of antidepressants on serotonin turnover rate in brain nuclei and on the development of specific methods to measure biosynthesis of endogenous ligands for opioid receptors.

The work of Drs. Marco and Meek on the chlorimipramine and nomifensine induced changes in 5HT turnover may do much to clarify how antidepressants act and also has raised interesting possibilities concerning the organization of serotonergic neurons in septum and hippocampus.

Drs. Bohan and Meek have introduced the use of high pressure liquid chromatography for positive identification of endogenous enkephalin in intestinal and brain extracts, and for positive identification of enkephalin formation after incubation of tissue with radiolabelled amino acids. Their work with enkephalin in the intestine should be very helpful in unraveling the mechanisms of enkephalin synthesis and release.

SECTION ON MOLECULAR PHARMACODYNAMICS (Chief: D.L. Cheney, Ph.D.)

Using mass fragmentography, steady-state labeling with precursors containing stable isotopes of C and H and stereomicroscopic dissection of brain nuclei following microwave fixation of the brain, the turnover rates of ACh (TR_{ACh}) and GABA (TR_{GABA}) have been measured. These techniques have been developed in this section during the last few years. This year, Dr. Cheney and colleagues have studied how antischizophrenics, narcotic analgesics, anxiolytics, antidepressants and cannabinoids modify the dynamics of several cholinergic and GABAergic pathways in rat brain. A change in the TR_{GABA} and TR_{ACh} can be used as an index of the changes in transmitter utilization elicited by various drugs. Since these drugs do not elicit generalized changes of TR_{ACh} and TR_{GABA} , the view has been entertained that the changes in transmitter metabolism derive from specific alterations in neuronal activity. Since this method specifically studies those pathways which are involved in the action of drugs used in psychiatry, the measurement of TR_{ACh} and TR_{GABA} has become a method to assess modifications of the postsynaptic receptor activities caused by the drugs. The drug induced modifications of TR_{ACh} and TR_{GABA} are now being tested by electrical stimulation of the various brain nuclei. The necessary evidence to relate the changes in GABA or ACh turnover to trans-synaptic modulation of specific afferents is being documented also by local injections of specific receptor agonists and antagonists.

Opiates and Septal Hippocampal Cholinergic Mechanisms

Dr. Moroni has compared the changes in TR_{ACh} in brain structures of rats injected intraventricularly with morphine and the opioid peptide, beta-endorphin. The TR_{ACh} in cortex, N. accumbens, globus pallidus and hippocampus is decreased by both compounds, and this decrease is reversed by naltrexone. The observation that the TR_{ACh} in the hippocampus is inhibited by 85% and the analgesia is elicited after intraventricular doses of beta-endorphin which were 100-fold less than those of morphine suggesting that specific receptors were modulating cholinergic axon terminals in hippocampus. Since the hippocampus contains the terminals of cholinergic neurons originating in septum, Dr. Moroni has further investigated the septal hippocampal pathways. He has found that intraseptal injections of morphine and beta-endorphin are not able to induce analgesia or catalepsy, but decrease the TR_{ACh} in the hippocampus. Further, intraseptal injections of naltrexone curtails the decrease of hippocampal TR_{ACh} caused by intraperitoneal morphine. These experiments demonstrate that the septum contains opioid receptors associated with the regulation of cholinergic cell body or dendrite excitability which probably are modulated by endogenous opioid ligands. These receptors are located either in the cell bodies of cholinergic neurons or in the axon of afferent neurons which excite the cholinergic neurons. Since the opioid

inhibition of hippocampal cholinergic system can be dissociated from analgesia, the possibility should be considered that endogenous opioid ligands function in brain also independently from pain control mechanisms.

Peptidergic Mechanisms and TR_{ACh} in Various Brain Structures

In this and other laboratories, other neuronal peptides have been shown to have nonuniform distribution throughout the brain and to be present in nerve terminals suggesting a possible role in neuronal transmission. Drs. Malthe-Sorensen and Wood have shown several of these peptides to specifically modulate the metabolism of ACh in neurons of several brain nuclei. These modifications may be indicative of the presence of peptide receptors in the outer membrane of cholinergic dendrites, cell bodies or axons. Physiologically these peptides are released from neurons afferent to cholinergic neurons; the peptides by activating receptors on cholinergic neurons modulate behavioral and physiological parameters. Dr. Malthe-Sorensen has shown that Substance P decreases the TR_{ACh} in hippocampus and septum while somatostatin, neurotensin and TRH enhances the TR_{ACh} in some brain areas although the pattern of increase differs for the various pituitary neurohormones. Angiotensin II decreases the level of ACh in the cortex but does not alter the TR_{ACh}. Such experiments are now being interpreted after obtaining EEG recording to exclude paroxysmal neuronal activity. They suggest the presence of specific receptor sites for peptides in different parts of the brain which play an important role in the regulation of cholinergic neurons.

Antidepressants and TR_{ACh}

Dr. Robinson has shown that antidepressant drugs as a group do not elicit any consistent pattern of changes on either the ACh levels or the TR_{ACh}. This is in line with the present belief that certain transmitter mechanisms are altered by some antidepressant but not by others. Desipramine and chlorimipramine decrease the TR_{ACh} in striatum and hippocampus but these values return to normal after chronic treatment. Iprindol and nomifensine increase the TR_{ACh} in hippocampus. Nomifensine also increases the TR_{ACh} in cortex and diencephalon. The action of nomifensine parallels that of amphetamine but not that of apomorphine. Dr. Robinson has injected phenoxybenzamine locally into the septum and has found that it blocks the increase in TR_{ACh} in cortex and hippocampus elicited by parenteral nomifensine or amphetamine. These experiments suggest that the increase in TR_{ACh} in hippocampus after the administration of nomifensine is mediated by the action of this drug on noradrenergic receptors which interact with the cholinergic neurons. Thus, Dr. Robinson's work excludes a direct action of the antidepressants on brain cholinergic systems.

Antischizophrenics and TR_{ACh}

Contrary to the antidepressants, the antischizophrenic drugs endowed with cataleptic activity are a unique class of compounds in their action on TR_{ACh} in striatum and N. accumbens. Independently, from their chemical structure, they all increase the TR_{ACh} of striatum and N. accumbens. How-

ever, noncataleptogenic antipsychotics endowed with anticholinergic activity fail to increase the TR_{ACh} in striatum and N. accumbens. These results suggest that cholinergic neurons of striatum and N. accumbens are regulated in opposite ways by muscarinic and DA receptors: the former increase ACh metabolism, the latter inhibit it. On chronic administration, cataleptogenic antipsychotics fail to change striatal and N. accumbens TR_{ACh} . Thus, the change in TR_{ACh} does not appear to mediate the therapeutic action but could be involved in those side effects which like the increased muscle tone exhibit tolerance.

Cannabinoids and TR_{ACh}

Some interesting preliminary experiments have been performed by Dr. Revuelta and Moroni on the effects of Delta-9-THC on the TR_{ACh} in various rat brain areas. She has found that delta-9-THC decreases the TR_{ACh} in hippocampus and striatum but not in cortex or diencephalon. The dose that can still decrease hippocampal turnover is lower than 0.5 mg/kg.

Anxiolytics and TR_{ACh} and TR_{GABA}

Diazepam and muscimol decrease the TR_{ACh} in cortex and diencephalon without altering the TR_{ACh} in hippocampus and striatum. Drs. Mao, Marco and Revuelta have shown that muscimol and diazepam inhibit the TR_{GABA} in nigra but not in striatum. Indirectly, these experiments suggest that diazepam may act through a GABAergic mechanism. This possibility is emphasized by the results obtained by Drs. Mao and Peralta showing that caudate stimulation increases the turnover rate of GABA in substantia nigra.

Antischizophrenics and TR_{GABA}

Drs. Mao and Peralta have demonstrated that antipsychotics whether given in a single dose or in repeated injections enhance GABA turnover rate in N. accumbens. Yet only noncataleptogenic antischizophrenics increased the GABA turnover significantly in substantia nigra and N. caudatus. These results suggest that GABAergic neurons in N. accumbens may play a role in the therapeutic action of antischizophrenics that block DA receptors. Moreover, the action of noncataleptogenic antischizophrenics on TR_{GABA} in substantia nigra suggests that the noncataleptogenic antischizophrenics fail to cause extrapyramidal side effects because they indirectly block the input of GABA to substantia nigra. Nucleus accumbens perhaps contains DA terminals which innervate GABA neurons, and these synapses do not develop the phenomenon of tolerance discussed above for DA receptors on ACh cells of N. accumbens and striatum. The reason for such resistance is not clear at this time.

SECTION ON BIOCHEMICAL PHARMACOLOGY (CHIEF: N. H. Neff, Ph.D.)

The research of this section in general is directed towards understanding the biochemical events that preceded and followed the activation of biogenic amine containing neurons, i.e., serotonin, norepinephrine and DA. During the past fiscal period, we made significant strides toward our goal.

Dopamine Pathway in Spinal Cord

Of prime importance was the observations of Drs. Commissiong and Galli that dopaminergic neurons exist in the spinal cord. These neurons respond to neuroleptic drugs similar to the dopaminergic neurons of brain. The presence of these neurons in the spinal cord will undoubtedly change the current view that side effects and toxic effects of neuroleptics originate solely in the brain. The physiological importance of this new system is currently being studied by Dr. Commissiong.

Tyrosine Hydroxylase Regulation in Retina

Work from this laboratory has shown that neuronal activation of dopaminergic neurons is associated with activation of tyrosine hydroxylase. Enzyme activation is manifested as an increase of the affinity of the enzyme for a pteridine cofactor. This hypothesis, however, has never been assessed directly in vivo because of the lack of a responsive physiological system. Dr. Iuvone has discovered that retinal tyrosine hydroxylase is activated by light. In concert with the activation of the enzyme is increased dopamine formation. These studies are leading to a better understanding of tyrosine hydroxylase and its role in neuronal events.

Action of Cholera Toxin on Brain Serotonergic Mechanisms

Adenylate cyclase activation by cholera toxin can be used to biochemically activate dopamine containing neurons. Dr. Neckers (Lab. Clinical Psychopharmacology, NIMH) has shown that the toxin can be used to activate serotonin systems of brain for several days if it is injected into the raphe nuclei. Activation is observed as an increase in the rate of formation and disposal of serotonin and 5-hydroxyindoleacetic acid. Moreover, he has shown that activation can proceed in an apparent retrograde manner, that is, injection of toxin into the caudate nucleus results in activation of the dorsal raphe nucleus and not in the medial raphe nucleus, which does not project to the caudate. In addition, he has observed that long term activation of adenylate cyclase need not occur for activation of serotonergic neurons.

Dr. Chuang and Ms Seaborg have investigated the biochemical events that may provide the basis for activating adenylate cyclase by cholera toxin. They found that the A unit of toxin possesses nicotinamide adenosine diphosphate hydrolyzing activity and during the process of hydrolysis the A unit of the toxin becomes covalently bonded to adenosine diphosphate ribose. With other toxins, ribosylation of an endogenous protein is thought to be responsible for their biological activity. Studies are currently underway to see if ribosylated A unit is the substance responsible for activation of adenylate cyclase by cholera toxin.

Mass Fragmentographic Assay of Tyrosine Biosynthesis

Individuals who are heterozygotic for phenylketonuria are not easily

detected by the usual clinical procedures. Dr. Galli and Dr. Commissiong have been successful in developing a mass fragmentographic method for analyzing phenylalanine and tyrosine. With these procedures they have provided a method for evaluating the in vivo hydroxylation of phenylalanine which utilizes $^{18}\text{O}_2$ for detecting phenylalanine hydroxylase activity. Subjects breathe $^{18}\text{O}_2$ which is incorporated into tyrosine by phenylalanine hydroxylase. Individuals deficient in this enzyme form less ^{18}O -tyrosine. This is a simple test that should be of significant value for identifying heterozygotes for phenylketonuria.

SIF Cells and Sympathetic Ganglion Function

Sympathetic ganglia of rat have small intensely fluorescent cells (SIF cells) that contain DA which is thought to modulate the transfer of information from preganglionic cholinergic neurons to postganglionic sympathetic neurons. Dr. Luthold (Guest worker from Switzerland) is studying the biochemical pharmacology of DA in the superior, middle-inferior and celiac ganglia. He found that dopamine synthesis is enhanced by stimulation of muscarinic receptors and inhibited by stimulation of nicotinic receptors. Moreover, the celiac ganglion is the most responsive ganglion studied to activation by carbachol. These studies are leading to a better understanding of the basis for dopamine's ability to modulate synaptic events.

Monoamine Oxidase Inhibition

Monoamine oxidase (MAO) inactivates the biogenic amines and, therefore, is an important enzyme for controlling the concentration of these amines in neuronal tissues. MAO inhibitor drugs are used clinically as antidepressant and antihypertensive agents. We have studied the properties of cardiovascular and brain enzymes in an attempt to understand their physiological roles. We found that cardiovascular tissue amine oxidases are quite different from those of brain. Pargyline, an MAO inhibitor, was not capable of inhibiting all the enzyme activity in cardiovascular tissue but it was capable of inhibiting the enzymes of brain. Moreover, the types of enzymes associated with cardiovascular tissue were different from those of brain. A better understanding of the MAO enzymes could lead to more effective drugs for the treatment of depression and hypertension.

SECTION ON NEUROENDOCRINOLOGY (CHIEF: A. Guidotti, M.D.)

The goal of the section is to study the transsynaptic regulation of second messenger responses in various systems and the role of the second messenger responses for short and long term enzyme adaptations. Special emphasis was given to interactions among various transmitters in the regulation of hypothalamus, adrenal medulla, pituitary, and pineal.

Role of GABA and DA Receptors in the Feedback Regulation of Striatal Tyrosine Hydroxylase

Support for the involvement of DA in the etiology of mental disorders comes from the finding that drugs that block DA receptors cause a symptomatic relief of schizophrenia. All antischizophrenic drugs increase the firing rate of the DA neurons, accelerate the rate of formation of DA and produce

an allosteric activation of tyrosine hydroxylase in these structures. Since these drugs act on tyrosine hydroxylase in vivo but not in vitro, their action is indirect. Dr. Gale investigated by which receptor mechanisms antipsychotic drugs the kinetic properties of tyrosine hydroxylase activate in the nigrostriatal system. She and Dr. Toffano (Guest worker from the University of Padova, Italy) found that discrete lesions of neuronal pathways descending from striatum to substantia nigra (SN) abolish the ability of antischizophrenics to cause activation of striatal TH. These lesions resulted in a 60-90% decrease in GABA and substance P content in SN without damaging DA containing neurons. Injection of GABA mimetic compounds such as muscimol or diazepam, either systemically or directly into SN, blocked the antischizophrenic induced activation of striatal TH. On the other hand, systemic administration of GABA antagonists, such as picrotoxin or isoniazid, potentiated the action of haloperidol. The data suggest that GABA receptors and possibly also substance P receptors in SN, play a role in the mediation of the effect of antipsychotic drugs on the activity of nigrostriatal DA neurons.

Involvement of GABA receptors in the action of minor tranquilizers

The mechanism of action of benzodiazepines has long been unknown. Previous work had suggested that benzodiazepines may exert their action via the GABA system. Drs. Biggio, Naik and Grandison have found that muscimol and nipecotic acid delayed the onset of convulsions induced by isoniazid in doses that did not protect the animals from strychnine convulsions. Moreover, diazepam and muscimol selectively protect the increase of cerebellar cGMP or pituitary cAMP induced by isoniazid, picrotoxin and are virtually inactive in antagonizing harmaline and reserpine. Since GABA receptor activation regulates the second messenger response of cerebellum, hypothalamus and pituitary in a fashion very similar to the effects of diazepam, it was inferred that some of the pharmacological action of the benzodiazepines may be mediated by an effect on GABA receptor function. In line with these results, Dr. Gale has shown that in the nigro striatal system, the benzodiazepines, like other GABA receptor agonists, block the activation of DA neurons induced by the antipsychotics.

Opiate Receptors and the Regulation of Dopaminergic Function

Drs. Biggio and Gale have shown that analgesic doses of morphine and viminol R₂ increase striatal dopamine turnover rate. This increase of DA turnover is unrelated to an increase in the firing rate of dopaminergic neurons or to a blockade of postsynaptic DA receptors. However, morphine blocks the activation of striatal TH induced by antischizophrenic drugs. Morphine, viminol R₂, axidomorphine increase cGMP content in striatum and N. accumbens but decrease that of cerebellum. The increase of cGMP content in striatum and in N. accumbens and the decrease of cGMP content in cerebellum caused by systemic injections of narcotic analgesics are blocked by naloxone. Thus, an increase in the cGMP content in striatum can represent a second messenger response related to the interaction between opiate receptors and their agonist. The location of the opiate receptors and the relation of opiate receptor function with the DA neurons in substantia nigra and striatum is now under investigation.

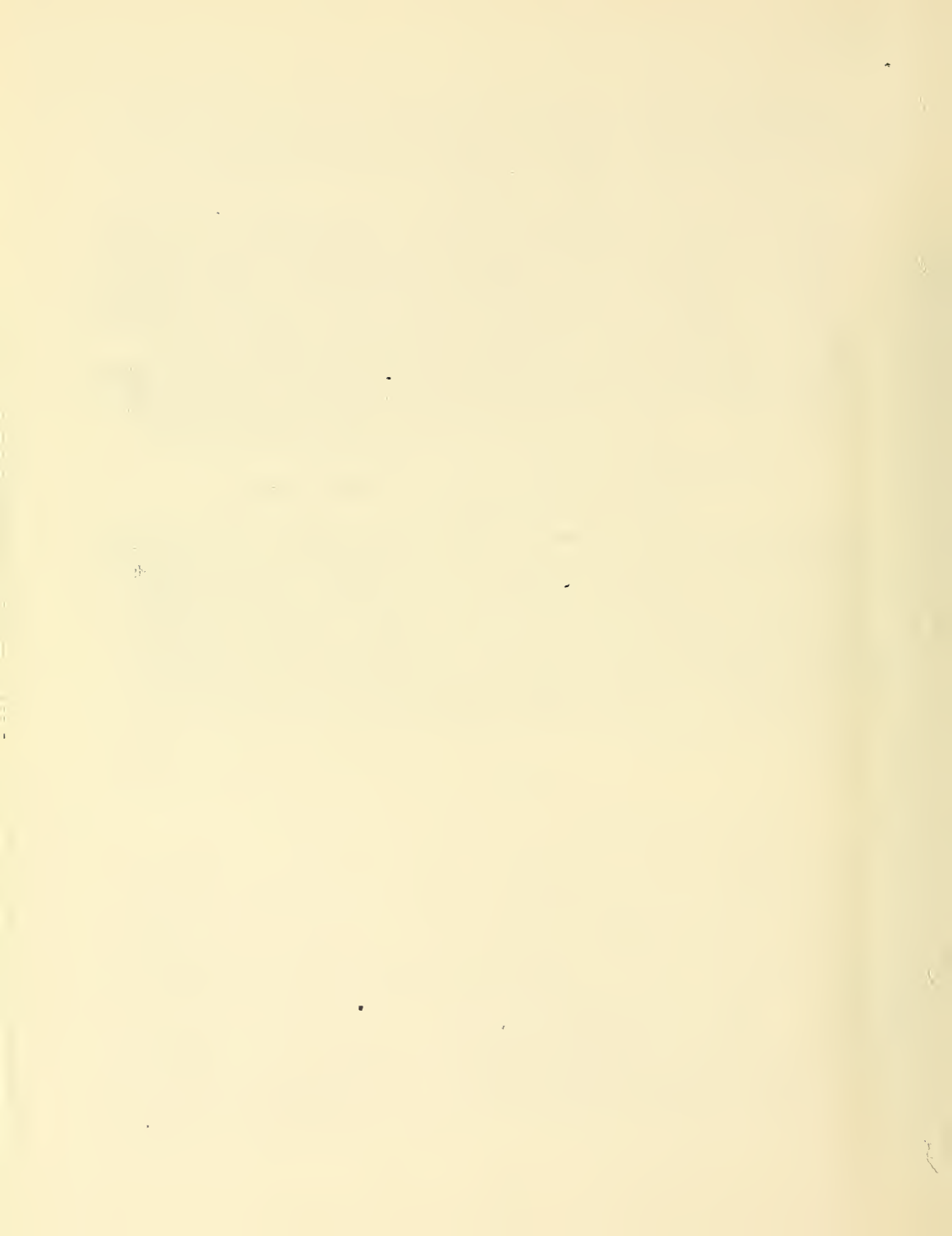
Role of Nicotinic Receptors in the Transsynaptic Control of

Protein Synthesis

Dr. Kurosawa has shown that in chromaffin cells of adrenal medulla, the following sequence of molecular events occurs in response to the persistent activation of nicotinic receptors: an increase of the cAMP concentration; activation of cytosolic cAMP-dependent PK translocation of the low molecular weight subunit of PK from the cytosol to the nuclear fraction and induction of tyrosine hydroxylase. Transection of the splanchnic nerve after the increase of cAMP has occurred and the cytosol protein kinase has been activated and translocated to the nucleus prevents the induction of tyrosine hydroxylase. Evidently, the function of nicotinic receptors controls the metabolic changes in RNA transcription caused by the translocation of PK in the nucleus. The way in which the sequence of the molecular events leads to tyrosine hydroxylase induction is being studied.

Role of cGMP Dependent Protein Kinase in the Expression of GABA Receptor Activation

Dr. Szmigielski has discovered, purified and characterized a thermostable small molecular weight protein in rat brain. This protein inhibits cAMP, cGMP and cyclic nucleotides independent protein kinase. This endogenous protein is particularly concentrated in rat cerebellum, the levels are transsynaptically regulated by stimulation of GABA receptors. Conditions which produce a long lasting increase in the function of GABA receptors are associated with dramatic changes in the content of the inhibitory protein. We are now investigating the molecular mechanisms that produce these changes in this potent regulation of phosphorylation.



Annual Report of the Section on Technical Development

National Institute of Mental Health

National Institute of Neurological and Communicative Disorders and Stroke

July 1, 1976 - September 30, 1977

Theodore R. Colburn, Ph.D., Chief

The Section on Technical Development is a group of engineers, computer specialists, and technicians which provides technical services to the Intramural Research Programs of NIMH and NINCDS. A veterinarian and a group of laboratory animal technicians and animal caretakers provide animal medicine and care services to the Intramural Research Program of NIMH. The major functions of the Section are:

(1) Instrumentation research and development. Design and development of instruments and instrumentation systems which represent advances in the state-of-the-art. Most of the research within the Section falls in this category, and is generally done in collaboration with investigators in the laboratories of NIMH and NINCDS.

(2) Production of custom instrumentation. Design and fabrication of electronic, mechanical, and optical equipment to suit the particular needs of the requesting investigator. These instruments, while often quite complex, utilize rather than advance the current state-of-the-art in design techniques and components.

(3) Computer services. The Section assists the investigators in data collection, reduction, and analysis, by supporting two laboratory digital computers for general use, including real-time on-line applications, and by providing programming service and technical consultation.

(4) Laboratory animal medicine and care. The Section provides veterinary medical care and a centralized program of animal caretaking functions. A health care program for employees who work with animals is being developed.

Additional services provided by the Section include consultation on measurement techniques, signal processing, noise and electro-magnetic interference in data measurement systems, and equipment purchases. Several formal and informal courses for investigators are taught by Section personnel; topics include electrical circuit theory, operational amplifier applications, digital logic design, and computer applications.

Due to manpower limitations and economic considerations, the Section is unable to provide the following services: repair of commercial instruments, duplication of off-the-shelf commercially available equipment, and fabrication of non-instrument items (shelves, bookcases, etc.).

When an investigator requires the services of the Section, he first meets with the Section Chief and other personnel as needed to discuss his requirements. On the basis of this meeting, a decision is made as to whether the Section will take on the project. In some cases it is more appropriate that the work be done elsewhere, due to the nature of the project or to an excessive backlog of work.

When the Section Chief agrees to take on a project, the investigator is required to submit a memo, initialed by his Lab Chief, which formally requests the services of the Section and provides complete specifications of the functions which are to be performed by the required instrumentation. Section personnel will respond in writing as to how the instrument is to be built, how much it will cost, and approximately when it will be ready. The Section does not charge for services, but the investigator will be billed for the cost of the components used. Upon delivery of the completed instrument, a memo is sent to the investigator listing the component costs and asking permission to have the Administrative Officer transfer funds from his CAN to the Section's CAN.

Two essential changes have been made this year in the operation of the Section. Short-term mechanical jobs (four hours or less), which have an emergency nature such that an on-going research project is being delayed, are now being handled on a first-come, first-served basis by a technician assigned to that duty for a two week period. This service has been instituted so that an investigator need not wait two or three weeks (our typical backlog) to have a small job done if his research will be delayed. However, we wish to stress that this service is provided only for urgent projects, and must not be abused by persons who do not properly anticipate their instrumentation needs, or simply wish to circumvent the system.

The second change involves the auxiliary machine shop. Although the Section has traditionally provided a facility for investigators to do their own mechanical work in an unsupervised situation, this policy was reconsidered in light of the frequent abuse of the machinery (generally due to inexperience and haste) and disregard for minimal safety and cleanup standards. We now station the technician responsible for small jobs in the auxiliary machine shop; in general, he will do the work formerly performed by the investigator. In certain cases where the investigator insists on doing the work himself, and the technician feels that he is competent to do so, he will be allowed to use the machines under the supervision of the technician.

INSTRUMENTATION

The following are selected instrumentation projects undertaken during the past year. These are chosen from a total of 245 projects, and are representative of the types of electronic instruments and systems developed by the Section:

(1) Patient Activity Monitoring System. The Section has continued to develop a system for monitoring the activity patterns of ambulatory subjects. The system is built around the basic activity monitor, which was redesigned

this year. As the device is now configured, a piezoelectric accelerometer generates a voltage when the limb to which the monitor is attached moves. If the voltage exceeds a preset threshold, an event is recorded. A standard timing period (generally 15 min.) is divided into 4096 equal intervals of 0.22 seconds, and only one event can be recorded in each interval. At the end of the timing period, the total number of events, divided by 16, is stored in solid state memory; the event counter is cleared, and a new timing period is initiated. The memory can store activity totals for 256 timing periods (64 hours), and the range of activity counts for each period is $4096 \div 16 = 256$.

Stored activity data is extracted from the monitor by reading the device directly into a PDP-11/40 computer through interface electronics. The computers are located in Building 36, Building 10, and the William A. White Building at St. Elizabeth's. With the computer handling the read-out, permanent storage, numerical processing, and plotting of the activity data, the need for manual data handling has been essentially eliminated. This development has been a major factor in the success of the activity monitoring programs thus far.

Other new developments in the system include:

(a) Portable Readout and Storage Device. In order to facilitate use of the monitors with subjects in remote areas, where access to the computer for data readout is impractical, a device was built into a briefcase to provide interim data storage. The device, designed and built to be operated by the subject, reads the data from the activity monitor and stores it on an audio cassette tape in a recorder which is concealed from the subject. Each tape holds about one year of data. The briefcase is returned to the investigator, who then plays the audio tape directly into the computer.

(b) Data Transmission by Telephone. An equally promising but potentially cheaper method of transporting data from remote locations is now under development. The device will read data from the activity monitor and transmit it by frequency shift keying over telephone lines to the investigator here at NIH, where the data will be stored on audio cassette tape until it can be read into the computer. Thus, long term outpatient studies can be conducted in any area with telephone service.

The activity monitor system has been well received by the technical community; over 35 monitors are now being used by seven different research groups. Applications include research on manic-depressives, hyperactive children, anorexia nervosa patients, drug addicts, and restrained monkeys. Publicity gained from word-of-mouth and from our two publications has evoked numerous inquiries from investigators all over the country. We have filed a patent application, and several firms have expressed interest in licensing the device for manufacture.

(2) Measurement of Activity of Confined Animals. The Section has continued to innovate techniques of transducing and recording quantitative and qualitative aspects of confined or restrained animals. The following are examples.

(a) Rat Activity Monitoring Cage. An activity cage has been developed which electronically senses the location of an unrestrained rat

within the cage, the amplitude and frequency of gross and fine movements, and the occurrence of rearing. The instrument produces two analog voltages which together contain this spatial and temporal activity information.

Four capacitors are created by mounting conductive plates on the outside of each wall of the cage. The electrically grounded conductive floor of the cage is the common second plate of all four capacitors, which form a differential capacitor pair for each axis, X and Y, of the floor of the cage. These capacitors serve in the timing circuits of four one-shot multi-vibrators. As the rat moves within the four overlapping capacitive fields, he changes the effective size of each capacitor and thus, the period of each one-shot. Additional circuitry detects the period changes and produces the two analog output voltages, which indicate the instantaneous position of the rat in the X and Y axes.

One system is being developed which will include fifty of the activity cages interfaced to a PDP-11/03 microcomputer for data acquisition. Programs in the 11-03 will analyze the output signals from the cages in real time for types of activity pertinent to a particular study. Summaries of that information will be recorded on a digital cartridge tape unit. The cartridges will be transported to a larger computer to be read for further analysis.

(b) Measuring and Recording Long Term Rat Activity. A micro-processor based system for continuous monitoring of activity from 16 rat exercise wheels was developed. One full rotation of a wheel sets an appropriate flag bit; every ten seconds the microprocessor scans all 16 flag bits and stores the data on a digital tape cassette. Data analysis of the activity patterns is done off-line on the Section's PDP-11/40 computer. Programs were written to present the data in the classic format currently used for similar data taken from mechanical event recorders.

(c) Activity of Monkeys in Restraining Chairs. A system is being developed for periodic measurement and recording of activity and temperature data from as many as 12 monkeys in restraining chairs, for purposes of observing physiological rhythms. A thermistor affixed to the cortex transduces temperature, and a specially designed activity monitor mounted to the skull measures head movement. The data from all animals is periodically acquired by a microprocessor and stored on digital cartridge tape, for later analysis on a digital computer.

(d) Rat Rotation Monitor. This device transduces and counts the clockwise and counterclockwise rotations of a rat for neuropharmacological purposes, such as evaluation of the efficacy of catecholamine lesions. Four channels are provided with a single, central timing control for simultaneous processing of four animals.

Each animal is harnessed by a combination Velcro waistband and wire neckloop via a stiff spring to a mechanical-electrical interface. This interface converts each revolution to three distinct electrical pulses by means of slotted, optical-limit switches. The interface is positioned at the top center of the animal's vertical cylinder plexiglas cage.

The device encodes the three pulses to recognize only full revolutions and stores these revolution counts in two counters, one each for clockwise and counterclockwise. Total counts are summed for user selected intervals of five or ten minutes. Each count is sampled at ten second intervals, converted to an analog signal and output to the user's recording device, typically a strip chart recorder. An alternate digital output mode is

available: each count pulse is presented at the time it occurs. This mode is TTL compatible.

(3) Event-Centered Raster Generator. This instrument detects a random event, such as a voluntary animal response, and produces a dot-intensified raster display that is centered in time on that event. Equal time segments of pre-event and post-event neuronal responses may be observed for up to 64 sequential events. For each raster sweep, the neuronal responses are displayed within 256 time bins, with the bin time selectable from 1 to 99 msec. LED displays show the number of raster sweeps and the total number of both pre- and post-event responses.

A secondary event, either synchronized with or independent from the primary event, may also be detected and displayed on the raster. Thus, for stimulus-response experiments, the raster may be centered on either the stimulus or response and the time correlation of neuronal responses to both of these "events" may be recorded.

(4) Discriminator and Iontophoresis Systems. The amplitude/time window discriminator system designed in 1973 (usually including an amplifier/filter, a raster generator, and a rate meter), and the multichannel high-voltage iontophoresis system designed in 1974 continue to find wide use. This year five discriminator systems and two iontophoresis systems were completed. These units bring the total number in use in the Intramural Research Programs of NIMH and NINCDS to 19 discriminator systems and 8 iontophoresis systems.

(5) Microelectrode Preamplifier. The latest IC operational amplifiers have been used to develop a battery-powered preamplifier for unit recording with metal or glass microelectrodes. The device has high input impedance, low noise, and selectable gain and bandwidth.

(6) Respiration Monitor. A previously developed respiration monitor for monkeys was improved by the use of a smaller and more sensitive thermistor. The thermistor is mounted in a small plexiglas protector and is positioned near the animal's mouth or nostrils to sample the inspired/exhaled air. This position generally requires the animal's head to be immobilized. The output can be either to a meter for qualitative observation or directly to an EEG recorder. Amplification is adjustable so that satisfactory results have been achieved for infant monkeys during surgery or adults in experimental situations. Circuitry can be included to sound an alarm when breathing ceases for a preset time interval.

(7) Rat Sleep Scorer. A device is being developed to categorize the bifrontal electrocortical activity as low, medium or high, the frontal-occipital EEG as low or high and the EMG as low or high. It is hoped that logical combination of these states will enable the user to identify and record 10 second epochs of sleep as wakeful, REM, or NON-REM.

(8) Finger Tremor Transducer. A device to detect and quantify the finger tremor of human patients with Parkinson's disease is being developed. Measurement of differential capacitance changes modulated by thumb or index finger movement will accurately locate and track the finger position and movement. Outputs will be analog for input to a strip chart recorder.

(9) Optokinetic Nystagmus Stimulator. This device was constructed for use with adult monkeys in restraining chairs. The unit consists of a 90 cm. diameter drum, support framework, a DC motor to rotate the drum, and a control box. The support frame and drum rest atop the animal's chair for testing and are easily removed. The inside of the drum is unobstructed for easy attachment of visual stimuli. Interval illumination is provided.

Controls permit selection of rotational direction and choice of several discrete speeds between 5 and 40 RPM. The control box is self-contained, allowing remote use.

(10) EMG Biofeedback Device. A prototype EMG biofeedback device was designed and constructed to be used in muscle reeducation for post-stroke patients. Although the device can be used for any EMG biofeedback application, it was specifically intended for use in reeducation of leg muscles. The device can be used in several different ways. A microammeter is provided which can give immediate feedback for training purposes; the movement of the meter dial is proportional to the EMG activity. The device can also be used for long-term feedback where the total EMG activity is integrated for the desired period of time up to approximately eight hours. A small digital display gives the user a number which represents the total integrated EMG activity over the selected time period. In this mode, the patient would wear the device; therefore, the instrument had to be small, portable, battery powered and easy to use.

(11) AR-11 Interface. The PDP-11 computer is equipped with a real-time analog subsystem that is used by many investigators for input and output of analog data to their computers. The AR-11 includes A to D and D to A converters plus a programmable real-time clock. Five interface panels were designed and constructed to take full advantage of the AR-11 capabilities and in some cases modify the output signals for use with specific scope displays. The interface panel makes possible easy access to the 16 channels of A to D provided by the AR-11 and also allows the use of the digital control lines that are available for the display scopes. In addition, multi-use Schmitt-trigger circuitry is provided that makes use of one of the system's interrupt control lines.

(12) Electromagnetic Muscle Stimulator. A prototype device was developed to provide mechanical stimulation of individual muscles in an intact awake behaving primate. A 3 x 6 x 15 mm steel bar is implanted longitudinally in the flexor carpi ulnaris muscle of the forearm. The monkey sits in a primate chair with the forearm passing through a solenoidal coil of wire. A pulse of current passed through the coil creates an axial magnetic field which produces a force on the implant and results in a mechanical stretch of the muscle, followed by a tendon jerk and a transcortical response. Further development of the system is directed toward increasing the force and minimizing the required input power, and in using various time varying currents for different effects.

(13) Data Acquisition System for Behavioral Observation. A micro-processor based cassette data entry system has been developed for use in behavioral observation experiments. The system utilizes two custom keyboards which observers will use to record behavioral events. The keyboards

have illuminated displays which indicate current behavioral trends or patterns. A microprocessor sorts, time correlates and writes keyboard data onto a digital magnetic tape cassette. Following the observational period, the tape is removed to a computer facility for information retrieval, processing and analysis.

COMPUTERS

The laboratory computer system developed by the Section on Technical Development provides a versatile means of computer support to the individual laboratories. The computer, as used in the Intramural Program, is a laboratory instrument, providing a means of data acquisition, both off-line and on-line, process control and data reduction. The laboratory systems applications range from real-time systems interacting with the experiment to reading the data stored in the memories of the Patient Activity Monitor. The smallest system consistent with the need of the laboratory is used, and data is further processed on STD's support computers or the DCRT facilities.

The aim of the Section has been to provide routines specifically tailored for the needs of the intramural program, and to furnish systems assistance to the investigators writing their own programs. The Section provides individual training and assistance to users. This has proven to be a more efficient means of training than conducting formal classes.

The installation and implementation of the laboratory computers has been well within the predicted time frame, and the Section is now exploring means of improving the resources available to the investigator and to lessen the investment of time necessary for a scientist to efficiently utilize the computer as a laboratory instrument.

Programming is now done primarily in FORTRAN. FORTRAN is a relatively old compiler that was designed to process mathematical equations. Computer manufacturers have standardized on this language, but it is awkward and not efficient for laboratory use. The language PASCAL has been purchased and is being studied in detail. It should reduce the amount of programming time needed and produce more efficient computer code. The concept of Structured Programming is being examined and its application to on-line, real-time programming should provide a more efficient means of handling complex programs as well as reduce the number of errors and the time spent debugging experimental routines. Methods of handling time series data are being investigated. There have been many recent advances in digital filtering, Fourier transforms and noise analysis which have direct application to neurophysiological data processing.

The Section conducted systems studies for the application of small microcomputers for fixed purpose, laboratory instrumentation. Two such systems have been ordered for the Laboratory of Clinical Psychopharmacology. One will provide a continual monitor of physical activity and brain temperature of a number of monkeys over extended periods of time. The other application will monitor the activity of rats living in the cages designed by the Section.

Additional computer-related projects include:

(1) Automated Analysis of Autoradiographs. An operator interactive system for the densitometric analysis of autoradiographs is being implemented for the Laboratory of Cerebral Metabolism. Under control of a PDP-11/03 computer, the information present in pictorial form in the autoradiograph is converted to digital densitometric data by a scanning microdensitometer and displayed on a video monitor. Standard image enhancement techniques, such as contrast stretching, density slicing and pseudo-color, in conjunction with a joystick-controlled outlining frame, will allow image features to be easily detected and isolated. Once a feature of interest has been isolated, the system will be able to compute its average density and area. A hard copy of the enhanced image may be obtained either by taking photographs from the video monitor or by using the Versatec printer/plotter, which is part of STD's Building 36 support computer.

(2) Membrane Activity of Neurosecretory Cells. Cells have been isolated that exhibit bursting pacemaker activity; some of these cells are responsive to peptide neuro-hormones. Voltage clamp experiments designed to investigate the conductances which produce the electrical responses are in progress. The data, currently stored in analog form on tape and analyzed off-line, consists of slow conductance variations combined with extremely fast changes during the bursting activity elicited by changes in membrane voltage. Digital techniques are being developed to separate and analyze these components in order to study the underlying mechanisms of chloride conductances and membrane permeability. The eventual aim of these programs is an on-line, real-time system allowing the scientist to interact with the experiments.

(3) Single Unit Studies. Hardware and software support of Laboratory of Brain Evolution and Behavior studies of single units in primate brain has resulted in the completion of the CAUDAR (Computer Assisted Unit Data Acquisition and Reduction) system. Personnel of LBEB developed the FORTRAN software package and the real-time data acquisition portion was developed by STD. The system has run successfully since June 1976 with continuing developments and enhancements up to the present time. It has been submitted to and accepted by National Technical Information Service.

LABORATORY ANIMAL MEDICINE AND CARE

The Unit on Laboratory Animal Medicine and Care was established this past fiscal year. The Unit is staffed by a veterinarian, laboratory animal technicians, and animal caretakers who provide daily care and maintenance of laboratory research animals.

Services provided by the Unit include:

(1) Tuberculosis testing program. All primates including those located at St. Elizabeth's Hospital are T.B. tested every three months.

(2) Manage Central Primate Facility. The Central Primate Facility

has a capacity for approximately forty primates. At the present time, three investigators have research projects in progress. Support is provided through assistance in preparing surgical models and maintaining animals on special diets and feeding routines.

(3) Treatment of sick and post-surgical animals.

(4) Assist in animal procurement when needed.

(5) Provide day to day care and maintenance of research animals. Care and maintenance of research animals are provided seven days a week in all areas. To assist in establishing and implementing work routines, a supervisor was appointed. The supervisor provides day to day contact with personnel in all work areas and coordinates weekend and holiday work schedules. A float position was established to provide animal care support when personnel are on leave.

Work routines that have been established include changing of all animal cages at least weekly. This has significantly reduced the incidence of gastrointestinal problems, especially in the primate colonies. Night coverage of the primate nursery has been established with the addition of two part-time persons covering on alternating nights.

(6) Organize and monitor an employee health program. This program will apply to all NIMH personnel who work directly with animals and/or animal tissues. It is designed to protect all personnel from zoonotic diseases and in turn to protect animals from human diseases. It will be jointly administered by NIMH Personnel, Occupational Medical Service (OMS) and Unit on Laboratory Animal Medicine. The NIMH Personnel Section will notify the OMS of new employees; once an employee is registered with the OMS, the service will notify the individual employee of recommended follow-up visits. A copy of these notifications will be sent to the NIMH Personnel Section and the Unit on Laboratory Animal Medicine as a means of keeping the employee list current with the Occupational Medical Service.

One of the major projects undertaken by the Unit is to upgrade the program's animal holding facilities. Over the next several years, it is anticipated that automatic watering systems will be installed in all rabbit and rodent rooms and on all cages. This will decrease the time needed to service each unit.

Automatic watering systems have been installed in three rodent rooms with two more rooms to be installed soon. Some new primate cages with squeeze mechanisms for easy restraint were purchased this year. New rabbit and rodent cages have been ordered to replace some of the older units that are no longer serviceable or did not meet size requirements.

Although the animal holding facilities at St. Elizabeth's Hospital are not part of the Unit due to logistic difficulties, the veterinarian is responsible for the quality of animal care delivered. Consultant visits are made approximately once a month to assist in care and maintenance of research animals and to take care of health problems that may exist.

ENGINEERING, COMPUTER and FABRICATION SERVICES

This table shows the distribution of the Section's workload among the various laboratories.

<u>LABORATORY OR BRANCH</u>	<u>HOURS</u>	<u>PERCENT</u>
Neurophysiology, NINCDS - - - - -	4774	15.29
Special Mental Health Research, St. E's., NIMH - - -	4059	13.00
Clinical Science, NIMH - - - - -	3196	10.24
Neurophysiology, NIMH - - - - -	3023	9.68
Brain Evolution & Behavior, NIMH - - - - -	2432	7.79
Adult Psychiatry Branch, NIMH - - - - -	1694	5.42
Behavioral Biology, NICHD - - - - -	1346	4.31
Experimental Therapeutics, NINCDS - - - - -	1342	4.30
Neuropsychology, NIMH - - - - -	1094	3.50
Cerebral Metabolism, NIMH - - - - -	1046	3.35
Neurobiology, NIMH - - - - -	975	3.12
Psychology and Psychopathology, NIMH - - - - -	959	3.07
Neurochemistry, NINCDS - - - - -	776	2.48
Central Nervous System Studies, NINCDS - - - - -	562	1.80
Developmental Psychology, NIMH - - - - -	544	1.74
Molecular Biology, NINCDS - - - - -	531	1.70
General and Comparative Biochemistry, NIMH - - - -	476	1.52
Biophysics, NINCDS - - - - -	464	1.48
Neurochemistry, NIMH - - - - -	392	1.25
Intramural Research Program, NIMH - - - - -	374	1.19
Neuro-Otolaryngology, NINCDS - - - - -	371	1.18
Experimental Neurology, NINCDS - - - - -	254	0.81
Neuropathology and Neuroanatomical Sciences, NINCDS -	222	0.71
Intramural Research Program, NINCDS - - - - -	82	0.26
Perinatal Physiology, NINCDS - - - - -	76	0.24
Infectious Diseases, NINCDS - - - - -	73	0.23
Medical Neurology, NINCDS - - - - -	66	0.21
- - - - -		
NIMH (Total)	20,264	64.94
NINCDS (Total)	9,593	30.75
NICHD (Total)*	1,346	4.31
	31,203	100.00

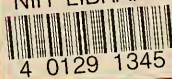
* NICHD loans the Section one position, and is thus entitled to 1700 hours of service.



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